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The empirical and systematic implications of the physiological - endocrine and neural - processes underlying an individual's experience with tinnitus are not yet fully understood. Individual differences in reaction to stressful situations, including tinnitus, can be detected in the autonomic nervous system as measured by alpha amylase, adrenal cortical secretions, as measured by cortisol, circadian cycles, as measured by melatonin, and the immune system, as measured by neopterin. These hormones contribute to how individuals experience tinnitus differently. The purpose of this study was to develop a model of the tinnitus experience with a focus on the influence of physiological changes in the endocrine and immune systems.

Ten male participants with tinnitus and ten male without tinnitus were exposed to an arithmetic induced-stress task. Saliva essays for cortisol, alpha-amylase, melatonin, and neopterin were collected. Behavioral and audiometric measures were administrated. Regression ANOVA models were used to examine any evidence of group difference on each of the four biomarkers, controlling for the effects of baseline measure, stress, sleep, and tinnitus severity. In addition, nonparametric tests were computed to control for the assumption of normality and homoscedasticity. The results suggest evidence of a potential difference in cortisol, alpha-amylase, melatonin, and neopterin reactivity in the tinnitus group. In addition, the results of this study demonstrate the feasibility of utilizing a psychological immune endocrinal (T-NPIE) model in the study of tinnitus.

THE DEVELOPMENT OF A NEURAL PSYCHOLOGICAL IMMUNE ENDOCRINE MODEL (T-NPIE) OF TINNITUS

by

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APPROVAL PAGE

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CHAPTER I

STATEMENT OF THE PROBLEM

Statement of the Problem

Chronic tinnitus is sound that is perceived in the absence of an external stimulus. According to Dauman and Tyler, chronic severe tinnitus, is a tinnitus that last at least five minutes and occur at least two times per week (Dauman and Tyler, 1992). An estimated 50 million Americans report they have experienced tinnitus at some point in their lives. Remarkably, 16 million Americans report having frequent tinnitus experiences. Overall, the experience of tinnitus seems to be positively correlated with aging (Sahrgorodsky et al., 2010). Based on the severity and nature of tinnitus, patients with chronic tinnitus may suffer from serious health conditions. For example, an estimated 28% to 45% of patients with chronic tinnitus show clinically relevant anxiety and distress symptoms (Anderson, 2004; Reynolds, 2004). Another more recent investigation shows a close association between tinnitus and a number of psychiatric disorders including anxiety and depression (Zoger, S., Svedlund J., & Holgers K., 2006). Yet, little is known about how the severity of tinnitus varies with any of these disorders. Chronic tinnitus can be an extremely handicapping and debilitating problem in adults, especially among the elderly. It is also a problem for young Americans who are increasingly exposed to high intensity environmental noise. Until recently, many hearing health-care providers had little to offer patients with tinnitus except to say they should learn to live with it.

However, new clinical research over the past decade has focused on tinnitus management (Jastreboff & Hazell, 1993) and on establishing new approaches to investigate the origins of tinnitus. To date, only a few clinics dedicated to the treatment of tinnitus exist in the United States. The lack of access to such support services results in reduced productivity and increased costs to those with the condition.

Stress can be caused by internal biological changes that result in homeostatic disturbances in several organ systems including the endocrine and immune systems. Homeostatic reaction to stress caused by tinnitus or ringing in the ears involves releasing stress-related glucocorticoids (GCs), which are hormones such as cortisol; however, recent studies have revealed that GCs are not the sole factor in the manifestation of a stress reaction. Other stress-related hormones include: Alpha-amylase, Melatonin, and Neopterin. Regardless of its origin, persistent stress can lead to serious health conditions. Persistent stress involves interactions between the hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic sympathomedullary adrenal system. These combine to create both a neural and hormonal cascade of changes that feed back onto the nervous system to create the experience of stress. It is likely that the experience of tinnitus both exacerbates stress reactions and stress reactions exacerbate the experience of tinnitus.

Different service models have been developed and successfully utilized to provide individuals with chronic tinnitus psychological counseling, tinnitus assessment, and sound therapy (Givens et al., 2003), such as tinnitus retraining therapy (Jastreboff and Hazell, 1993); however, the establishment and usage of a comprehensive tinnitus neural-psychosocial-immune-endocrinal model that incorporates different neural systems and

networks could assist health-care providers in identifying and providing individuals with chronic tinnitus different intervention and management options.

The context for this dissertation is that there is a need for a model of tinnitus that includes the involvement of endocrine, immune, and psychological processes in the experience and modulation of chronic tinnitus. The current investigation seeks to identify the role of non-traditional factors in the modulation of tinnitus. These non-traditional factors would include structures and functions that are not limited to the auditory system but also include the autonomic nervous system, immune system, and endocrine system. The current investigation will examine the role of the autonomic and endocrine systems as measured by levels of four stress-related hormones in healthy males with and without tinnitus.

Figure 1 is a representation of the framework of the T-NPIE model that will be utilized for this study. The T-NPIE originates from our understanding of how stress influences individuals. Under stressful situations, two systems are activated, namely: the endocrine and the autonomic nervous system. The endocrine systems and the autonomic nervous systems are connected via a brain small structure called the hypothalamus, which is divided into small nuclei. When activated, the brain and hypothalamus trigger a cascade of hormonal secretions via the hypothalamic-pituitary-adrenal-axis (HPA) such as: cortisol, and melatonin. Interestingly, excessive production of cortisol is known to lead to weakening of the immune system.

On the other hand, the autonomic nervous system (ANS) responses to stress are more immediate in nature, hence, activated almost immediately after exposure to stress.

Within the ANS two systems also help the body cope with stress: the sympathetic (SNS) and parasympathetic (PSN) nervous systems. The sympathetic nervous system response to stress via activation of adrenaline and noradrenaline secretions into the blood stream, while the mean goal of the SNS is to remove the stressor, the PSN aim is to restore the body to it is normal pre-stress status.

A simpler way to measure responses of the SNS is by testing levels of salivary alpha-amylase (sAA). Alpha-amylase is an enzyme that is involved in the breakdown of starch and carbohydrate, as well clearing of bacteria in the mouth and digestion. Thus, sAA is assumed to represent the immune system first line of defense. There is evidence that sAA also can be used as an indicator of inflammatory reactions such as in the case of appendicitis. In addition to sAA, melatonin as well as neopterin is considered as markers of immune system inflammatory reactions, particularly in cases of oxidative and chronic stress.

With that in mind, tinnitus is a condition that is not only chronic in nature but also known to cause stress. In the majority of cases, tinnitus is a subjective disorder. If this T-NPIE model is demonstrated to relate to tinnitus, then health care providers may be able to utilize any part of this T-NPIE model to objectively identify the immune, psychological, and endocrinological features of tinnitus.The T-NPIE model suggests that tinnitus includes a disruption in the autonomic nervous system (ANS), which can lead to changes in the underlying endocrine and immune systems and vise versa. A stress-inducing situation will stimulate the ANS, which, in turn, will activate a reaction in the endocrine system via the hypothalamic pituitary adrenal (HPA) axis. Reactions of the

HPA axis can ultimately induce a weakening in the immune system response to infections.

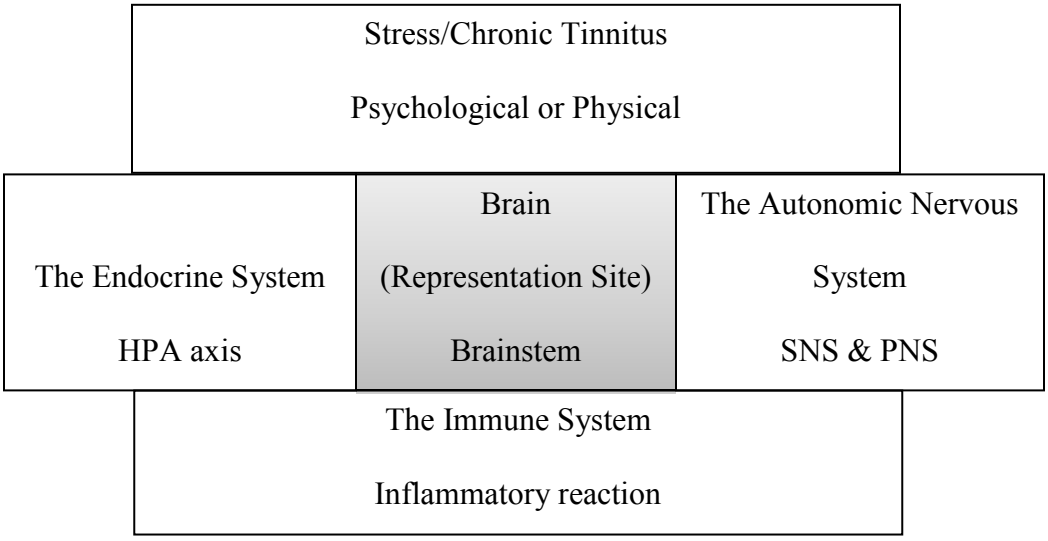

Figure 1. The Framework of the Neural Psychological Immune Endocrine (T-NPIE) Model of Tinnitus.

The aim of this study is to develop a model of the tinnitus experience, with a focus on the influence of physiological changes on the endocrine and immune systems, by assessing how the disruption of specific endocrine secretions and a weakening of the immune system contribute to the manifestation and experience of tinnitus symptoms. It has long been observed that patients vary in how disturbing tinnitus is to their daily lives. A baseline and three post- stress task samples of salvia will be taken to measure the levels of four stress-related hormones in healthy males with normal hearing and no tinnitus and males with tinnitus to determine if differences in these stress-related hormone levels can be detected in participants with chronic tinnitus. These samples will aid in determining if individuals react differently to stressful situations as indicated by their autonomic

nervous system, measured by alpha amylase, adrenal cortical secretions, measured by cortisol, and circadian cycles, measured by melatonin. This study will document if hormones contribute to individual differences in the experience of tinnitus. Results of this study may provide information about the experience of the person with tinnitus through the empirical and systematic examination of the underlying physiological processes, endocrine, immune, and neural. The goal is to better our understanding of tinnitus subsystems and possibly identify new sub-networks involved in the generation and perception of the tinnitus signal that are not limited to the auditory system, but involve the autonomic, immune, and endocrinal systems.

The long-term goal of this investigation is to:

1. Document whether specific subcortical hormonal areas influence the experience of chronic tinnitus by measuring the baseline of four stress-related hormone levels: a) Circadian cycle with melatonin, b) stress with cortisol, c) stress with alpha-amylase, and d) inflammatory immune system and neopterin to measure immune system reactions. It is common to have increased amounts of neopterin in individuals diagnosed with chronic health conditions; therefore, it is possible to find a similar association between markers of immune system activation, such as neopterin, in subjects with chronic tinnitus.
2. Investigate whether hypothalamic regulation of responses to stress, as reflected in the four stress-related hormone levels, is greater in male subjects with chronic tinnitus vs. those without tinnitus; and

3. Utilize the results to test a preliminary framework for a hypothetical T-NPIE model of chronic tinnitus that may lead to future investigations of underlying tinnitus factors as a consequence of the disruption of the autonomic nervous system (ANS), the hypothalamic pituitary adrenal (HPA) axis regulation of stress, and inflammatory reactions of the immune system.

CHAPTER II

REVIEW OF THE LITREATURE

A sound that is perceived in the absence of an actual external acoustic stimulus is known as tinnitus (Eggermont and Robert, 2004). Although tinnitus might be classified as a form of auditory hallucination, there is a "continuum of complexity" that is utilized to identify the true nature of each of these sounds (Wible, 2012) and, consequently, distinguish what is tinnitus or ringing in the ears and what may be a sign of a more serious health condition such as schizophrenia. Tinnitus is one of the most common phantom sounds a person can experience. Tinnitus perception may not be limited to simple sounds such as ringing, whistling, humming, hissing, buzzing, or clicking, but could include more complex sounds such as running water, breaking glass, wind, or even music. Despite the empirical and systematic examination of the endocrine and neural physiological processes underlying the tinnitus experience, the actual concerns of the tinnitus patient are not yet fully understood. The aim of this literature review is to shed some light on this complex phenomenon of tinnitus, paying special attention to the identification of systems and networks behind the emotional and psychological disturbances associated with chronic tinnitus. Accordingly, the following literature review will be divided into five main topics: 1) stress, 2) the hypothalamus, 3) the hypothalamic pituitary adrenal (HPA) axis, 4) stress-related hormones, and 5) tinnitus.

Stress

Stress History and Definition

The nervous system is continuously identifying and responding to perceived risks by sending signals to the brain to either react or suppress a response to a given situation. Stress can be considered as the state in which a situation is construed as being threatening or extreme—in which case the stress system and its components work together in an attempt to discontinue the stressful situation (Smith et al., 1989). Consequently, an accurate response to stressors is essential to survival. Alternatively, inadequate responses to stressors can results in disease or even death (Gold et al., 1988).

Humans strive to maintain a status of stability, best known as *homeostasis* (Chrousos & Gold, 1992). Changes in an individual's living environment and/or circumstances, whether physical, psychological, emotional, biological, or a combination of these, stimulate changes in that individual's internal homeostasis status and may cause biological alterations (McEwn, 2000). Claude Bernard was one of the first to propose the idea that the external and internal environment of an organism can influence its functions (Bernard, 1927). Consequently, the ability to keep an organism's internal environment steady in response to changes in the external environment is essential for understanding the impact that stress has on biological functions and life experiences. Bernard's views were adapted and further examined by Walter Cannon. Cannon was the first to introduce the term "homeostasis" in order to describe physiological reactions that maintain the steady state of the organism in the face of external stimuli changes (Cannon, 1932). According to Cannon (1932), homeostasis is a process of maintaining the internal

stability of an organism facing environmental changes. Cannon was also one of the first to observe the physiological and psychological disturbances that could elicit responses from the sympathetic nervous system and the adrenal cortex.

Another researcher, Hans Selye (1979), observed that the psychological and physiological changes of a stressor are mediated by the activity of the hypothalamic pituitary adrenal (HPA) axis. According to Selye, long exposure to a stressor of any type will cause various biological changes such as enlargement of the adrenal cortex, reduction of the weight of the thymus and lymph glands, and development of stomach ulcers. Selye was the first to introduce the term "stressor" to describe a source of threat. He also called these physiological responses "stress."

As a concept, stress has been extensively investigated because of its direct link to triggering physical, biological, and behavioral changes in individuals. Within recent years, a number of stress definitions have emerged, such as that of Bruce McEwen (2000), who defines stress as a real or interpreted threat to the physiological or psychological integrity of an individual that results in physiological and/or behavioral responses. Stress is also defined as a state of dissonance, or alarming homeostasis (Chrousos and Gold, 1992). Obviously, homeostasis is a core concept in the definition of stress. Furthermore, Steptoe (2000) suggests that stress affects four distinct domains: 1) physiology, 2) behavior, 3) cognitive functions, and 4) subjective experiences. Taken as a whole, a stress system is activated when a stressor of any type -physical, emotional, or psychological - surpasses a certain range or threshold. Figure 2 illustrates how activation

of a stressor above a certain threshold will unleash a cascade of behavioral and psychological changes.

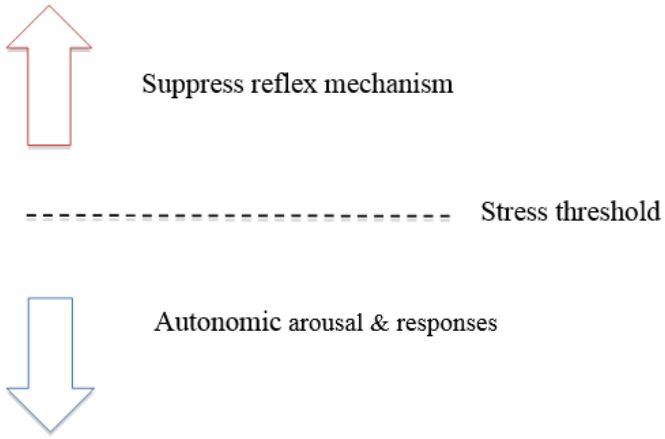

Figure 2. Reaction to Stress.

Stress Systems and Networks

Stress responses are modulated through highly sophisticated networks. *Stress network* is a term used to refer to a set of highly connected and conserved brain structures that are activated when real or false threats to an individual are perceived by an external source (Brown & Fisher, 1985). Activity in these networks, initiated by the amygdala, will trigger a cascade of sensory perceptions of pain, memory lapse, learning disruptions, and other disturbances. Additionally, heightened activity in the stress network will result in an increase in feelings of anxiety. Organisms respond to this state of stress and anxiety with behaviors that aim to reduce activity in the stress network (Pecoraro et al., 2006). One of the systems involved in the modulation of stress is the limbic system, which provides cortical knowledge of events and identifies the nature of the related stimuli. The limbic system has extensive connections to the ANS and is known to influence the

endocrine system. A second system is the hypothalamus, which regulates the majority of the endocrine system's features and is also regarded as a part of the limbic system. When a signal is identified as threatening or stressful, both the amygdala, and the hypothalamus are activated (Selye, 1976).

Prolonged Stress and Altered Hormonal and Neurologic Functions

Prolonged exposure to chronic stress, such as that experienced by individuals with chronic tinnitus, can lead to long-term changes in the HPA-axis activity, which include changes in glucocorticoid levels (Hebert and Lupien, 2007; 2009), abnormal responses to stressors (Selye, 1979; Chrousos and Gold, 1992; Habib, Gold and Chrousos, 2001), and impaired feedback regulation of HPA functioning (Chrousos, 2000; Selye, 1979). Finally, chronic stress can create changes in the brain and ultimately behavior patterns (Chrousos, 2000). While minor stressors can be brief and of limited duration, chronic stress is more sustained and has a repeated nature and/or intensity. Chronic tinnitus can be stressful. It would be logical to assume stress-related changes in the brain's neural networks may also be present in individuals with chronic tinnitus.

Stress and the Auditory System

Exposure to loud, continuous, or unexpected noises can be stressful for individuals. The effect of such exposure is often associated with auditory phenomena such as hyperacusis, sensitivity to sounds, and/or tinnitus, ringing in the ears (Jastreboff, 2000). Individuals attempt to maintain a steady internal environment; however, individuals with chronic tinnitus often experience stress associated with hearing a continuous, bothersome sound. In the case of chronic tinnitus, stress can reach a point

where it leads to more serious health conditions such as anxiety, depression, and sleep deprivation or insomnia. A chronic, continuous ringing in the ears can eventually lead to a series of physiological and psychological changes induced by stress (Hebert & Lupien, 2007). Similar to stress networks, a link between the central auditory pathways and the stress network exists. For example, through the medial geniculate body (MGB), the auditory system is connected to the limbic system that regulates emotions and behaviors (Kreibig, 2010; Sahley et al., 1997, Al-Mana et al., 2006). At the same time, the hypothalamus, which regulates activity of the ANS and the endocrine system via the regulation and modulation of hormones and neurotransmitters, is also connected to the central auditory system through the inferior colliculus (IC) (Al-Mana et al., 2006). Prolonged exposure to tinnitus can stimulate the stress networks and lead to a prolonged increase in HPA and ANS activity (Hebert & Lupien, 2007; 2009).

Conclusion

The impact stress has on the brain is of significant interest to many researchers for a number of reasons. First, examining the nature of stress allows researchers to identify stressors of psychological or physiological origin. Second, identifying the nature and characteristics of a stressor may aid investigators in building a stress-related disease model in humans. Third, observing how stress affects the brain may help explain how stress might alter human, behavior, and physiology. Continued exposure to stress alters a wide range of behavioral, emotional functiond in humans. The health consequences of chronic stress validate and strengthen the need for further detailed analysis and

examination of stress-related illnesses and conditions that may induce stress, such as tinnitus.

The Hypothalamus and the Nervous System

The hypothalamus, which is located on the wall of the third ventricle of the cortex, is fully functional at birth and considered to be the dominant influence on all emotions (Reeve and Reeve, 2001). The hypothalamus divided into three main regions: (a) the paraventricular, (b) lateral, and (c) medial hypothalamic.

There are several cells in the hypothalamus that secrete peptides, which are short chains of amino acids. Interestingly, a number of neurons in the hypothalamus project to regions of the nervous system as well as structures of the ANS and the limbic system (Saper, 2002). One of the main functions of the hypothalamus is the oversight of the endocrine system by secreting products directly into the general circulation of blood, via vasculature of the posterior pituitary, or indirectly by secreting regulatory-releasing and inhibitory hormones, which are carried to the anterior pituitary and affect its hormonal secretions. The hypothalamic-regulating hormones control the synthesis and release of the anterior pituitary hormones into the general circulation of blood (Harris, 1955).

The actions of hormones are specific. Certain hormones that cross the blood-brain barrier (BBB) or are released into the extracellular space or cerebrospinal fluid (SPF) will either activate or inhibit a restricted population of neurons. Such hormonal actions are involved in altering mood and behaviors (Harris, 1955).

The autonomic nervous system carries signals from the CNS to all organs and tissues in the body (Janig, 2006). Stress can trigger changes in ANS activity. ANS

activity is considered to be a major part of the regulation of different emotions such as anger, anxiety, fear, sadness, disgust, and embarrassment (Kreibig, 2010). Changes in ANS activity were also reported in depression (Hamer et al., 2007) and social anxiety (Haofmann et al., 2006). Although research has attempted to bridge the link between ANS activity in response to a variety of stress situations, the degree and specificity of ANS activity is very complex and not yet fully understood.

The Hypothalamus and the Auditory System

The auditory system acts as a warning system of possible danger or threatening sounds. The CNS is continuously assessing sounds that exceed certain intensity, frequency, or magnitude in order to identify the need for further precautions. The nature and meaning of a sound, its intensity, and its level are important parameters that the CNS detects to ensure a proper reaction. The CNS's ability to identify the origin and nature of a sound determines if that sound would be branded as a bothersome noise (e.g., tinnitus) or normal and acceptable (e.g., birdsongs). Exposure to continuous bothersome sounds, such as the wide range of perceptions experienced by individuals with tinnitus, may result in physiological changes in terms of altered homeostasis and CNS functions. The auditory system is connected indirectly, the non-classical pathway, to the limbic system via the medial geniculate body (MGB) and directly, the classical pathway, to other parts in the brain - the auditory cortex, the ANS, and the neuroendocrine system. Alongside these pathways are a number of connections between the auditory system and brain sites that also control and regulate emotions and physiological and behavioral responses.

Borg (1981) showed that sounds that are identified as bothersome or noisy activate the CNS, which initiates a number of physiological, emotional, and behavioral changes, most of which are beyond the control of the individual. Another investigations by Sahley, Nodar and Musiek (1997) look at a neurochemical model of tinnitus where endogenous dynorphins may induce hyperacusis or oversensitivity to sound. Endogenous dynorphins may also contribute to the induction, maintenance, and exhibition of tinnitus perception by altering auditory type I neural excitability via glutamate. Their findings indicated that stress-related neuromodulation may consist of products derived from hormones such as proenkephalin and prodynorphin. In yet another investigation, Hebert and Lupien (2007) examined stress regulation effects on tinnitus by assessing the reactivity of the HPA axis in patients with tinnitus when compared to healthy controls with no tinnitus. Their findings revealed that those with tinnitus showed a dull cortisol reactive pattern in response to psychological stress, suggesting that individuals with tinnitus might have some type of anomaly in regulating the HPA axis.

Conclusion

The hypothalamus plays an essential role in the regulation hormonal secretions that is involved in the expression of emotions (e.g. stress). The limbic system provides neural circuits that provide the underlying structures of emotions, such as anger and fear. Research in this area has expanded our knowledge of the connection and anatomical structures of the limbic system and its involvement in the regulation and modulation of stress. The afferent circuitry between the brain and the hypothalamus and the efferent connection between the hypothalamus and other structures are extremely complex.

Further research is needed to examine the activity in the hypothalamus in relation to chronic tinnitus.

The Hypothalamic-Pituitary-Adrenal (HPA) Axis

The HPA-Axis Secretions and Main Contributors

Secretions of the HPA are circadian in rhythm, with a noticeable increase in plasma cortisol during the hours before the onset of daily activity (Krieger et al., 1971). In humans, the magnitude of peak cortisol is linked to glucose requirements occurring towards the end of normal sleep periods (Oishi et al., 2005); however, upon waking, most individuals experience a further cortisol awakening response (CAR) usually 30 to 45 minutes later. These CAR responses are considered to be a reflection of the current load of stressors on the individual (Fries, Dettenborn, & Kirschbaum, 2009).

If identified as threatening, both physiological and psychological stressors can result in the HPA axis secreting adrenocorticotropin hormone (ACTH), Corticotropin-releasing factor (CRF), and glucocorticoid secretions (GCs) above a familiar range or threshold. Stress responses can be categorized in a way that involves the activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis, which in turn has seven main contributors:

1. Adrenocorticotropin hormone (ACTH)
2. The Corticotropin-eleasing factor (CRF)
3. The Corticotropin-releasing hormone (CRH)
4. Glucocorticoids (GCs)
5. The locus-ceruleus-norepinephrine (LC/NE)

6. The autonomic nervous system (ANS), and
7. The immune system

Because different types of stressors produce a wide range of behavioral, biological, and psychological responses, there are many factors to consider when determining the characteristics of a stress response. These factors include: 1) the type of stress induced, 2) the duration of a stressor, 3) the frequency of a stressor, 4) age, 5) gender, and 6) former stress experience of the individual exposed to the stressor. A dysfunction of the stress system or a malfunction of any of its components will result in cassation in a range of psychological and physical conditions (Habib et al., 2001). For the purpose of this study, the following section will only address some of the HPA axis networks, specifically glucocorticoids, the autonomic nervous system, and the immune system.

Glucocorticoids (GCs)

Glucocorticoids are lipid hormones secreted by the adrenal cortex that easily pass through the blood-brain barrier and influence mood, cognition, and sleep functions in humans. Glucocorticoids also play a major role in regulating and controlling HPA axis activity. GCs have an impact on the extra-hypothalamic regulatory centers such as the hypothalamus, frontal cortex, hippocampus, and the pituitary gland. They also play an important role in the termination of stress responses (Chrousos and Gold, 1992). In addition to its role in extinguishing the HPA response to stress, GCs have an inhibition function that limit the duration of the total tissue exposure to GCs secretions and, as a result, minimize the catabolic, antireproductive, and immune-supportive effects of these

hormones. The list below summarizes some of the main roles of GCs based on the work of Tsigos and Chrousos (1999, 2002).

1. Inhibition of CRH and LC/NE
2. Simulation of the mesocorticolimbic dopaminergic system
3. Inhibition of gonadotropin and thyroid stimulating hormones (TSH)
4. Direct insulin mediated effect on adipose tissue resulting in high blood pressure (hypertension) and insulin resistance
5. Direct effect on bone resulting in low bone density (osteoporosis)
6. Inhibition of inflammatory reaction, and stimulation of local inflammation
7. Prolonged CRH activation resulting in pathologies (e.g., melancholic depression)
8. Sexual insufficiency, irritable bowel syndrome, peptic ulcer diseases, and anxiety

Glucocorticoids are produced by the adrenal glands, which are small, enclosed organs embedded in fat and located above the kidneys. They are also known as the suprarenal glands. These glands have a slightly different shape and are located on each side of the body. The adrenal gland consists of two major parts: the outer cortex and the central medulla. The outer cortex is the part responsible for the formation of cortisol and a number of other hormones including steroids, aldosterone, and hydroepiandrosterone/DHEA. The adrenal cortex is the part that reacts to long and short-term stress, whereas the medulla is controlled by the nervous system and takes part in

rapid reactions to stress. The signals that trigger this activation, however, are believed to originate in the hypothalamus and reach the medulla by nerves from the ANS.

Glucocorticoids are essential for life and survival, as they act on different cells in the body; however, a prolonged chronic production of GCs, such as the secretion of cortisol, can lead to cell death, which may also result in immunodeficiency. Glucocorticoid has two main receptors known as type I and type II GCs receptors. Type I GCs receptors respond positively to low levels of GCs. They are also known as mineralocorticoid receptors (MR) because they respond to other secretions of the adrenal cortex. On the other hand, type II GCs receptors are considered to be the classic glucocorticoid receptors. In addition to the latter's role in responding to basal and stress levels, GCs type II receptors play a role in the negative feedback of an afferent GABAergic pathway to the paraventricular nucleus (PVN) (Chrousos, 2000).

The nature of glucocorticoids secretions. In non-stressed humans, cortisol secretion rates are between 8-25mg/day, and plasma concentrations range between 40-180 mg/ml, although the amount of plasma concentration varies depending on the time of the day. It is usually maintained within close limits (Lupien et al., 2009). GCs secretions (e.g., cortisol) are secreted in a pulsatile manner and are influenced by the circadian rhythm (Chung et al., 2011). manner and are influenced by the circadian rhythm (Chung, S., Son, & Kim, 2011).

Glucocorticoid secretions influenced by long and short term stress. Cortisol is secreted in large amounts in men during stressful conditions. As a result, prolonged and chronic stress will create higher amounts of circulating GCs. Long-term stress is often

more chronic in nature, while short-term stress is usually referred to as “alarming” and is often more sudden in nature. Sudden or short-term stress is regulated with hormones controlled by the adrenal medulla (e.g., EP). The adrenal medulla’s reaction to stress, on the other hand, is controlled by the central nervous system. Yet, both short and long-term stress results in the release of GCs (Tsigos and Chrousos, 2002).

Two types of stressors can play a role in the production of GCs. First, prolonged stress, such as the stress resulting from a brain injury or chronic tinnitus, evokes GCs production from the adrenal cortex. Second, acute and sudden stress, such as the stress resulting from an event of surprise or of alarming nature, certain fears or phobias, and even the onset of a tinnitus episode, will evoke the secretions of EP, NE from the adrenal medulla, and cortisol from the adrenal cortex. Interestingly, both type of stress usually occur simultaneously, suggesting that one chain of action does not stop or prevent the utilization of another.

The Autonomic Nervous System

Exposure to stress results in a series of coordinated responses. These responses enable the individual to react to a perceived threat in order to prevent or recover from it. The aim is to maintain a status of homeostasis or an internal steady environment. In the presence of a “stressor,” activities in both the ANS and HPA axis are increased. Changes in the ANS and HPA activity will also result in behavioral changes. Removal of the stressor usually terminates the response and the body reverts to its normal homeostatic state.

The ANS plays a major role in the regulation of important bodily functions such as temperature, digestion, respiration, metabolism, sweating, and aspects of the endocrine and immune system. The autonomic outflow is regulated by specific centers in the central nervous system. These centers receive input from a variety of ascending and descending central nervous pathways. A wide range of emotional, physical, and environmental stimuli and physiological changes influence these pathways. Stress can result in a wide spectrum of changes in the autonomic nervous system. For example, stress has been found to increase mean arterial pressure and tachycardia (Fisher, 2006) and immunosuppression (Padgett and Glaser, 2003) via the ANS.

The perception of stressful stimuli causes immediate activation of the sympathetic nervous system. The magnitude of the sympathetic responses depends on the nature of the stressor. Stress elicits a wide spectrum of behavioral responses depending on a number of factors such as the severity and duration of the stress as well as the individual's prior experience and coping abilities. In general, stress responses are adaptable and include activation of the neural pathways that mediate arousal and attention as well as avoidance behaviors. At the same time, chronic stress can inhibit the pathways involved in the regulation of growth, reproduction, and feeding. Not surprisingly, many of these behaviors are alarming and are indicative of anxiety.

In general, exposure to stress gives rise to a number of autonomic and behavioral effects. This might explain why a variety of mental and pathological disorders may occur following or during the exposure to chronic, severe, or uncontrollable stress.

The Immune System

Stress alters immune system functions via its effects on ANS and HPA activity (Padgett and Glaser, 2003). Alterations of the immune system function are known to lead to pathologies in humans and animals. Different types of stress, such as psychological and inflammatory, are involved in the activation of the HPA axis (Tsigos and Chrousos, 2002). Stress responses are capable of activating the central nervous system and the immune system together. As noted earlier in Table 1, activation of the HPA axis has an inhibitory effect on responses of the immune system (Tsigos and Chrousos, 2002). During stress, the autonomic nervous system exerts its own effect on the immune system, and this activity of the ANS can be either immune-supportive or immune anti-inflammatory (Elenkov and Chrousos, 2007).

The Hypothalamic-Pituitary-Adrenal (HPA) Axis and the Auditory System

Stress-inducing stimuli have been found to evoke a high rate of hormone secretions via the activation of the HPA axis (McEwen, 2000). Exposure to certain sounds, such as ringing in the ears or chronic tinnitus, can be stressful and may trigger the activation of the HPA axis. The CNS may react by distressing the equilibrium of several physiological functions. This type of high intensity or continuous exposure to bothersome sounds has biological effects on the body as well as the central nervous system. Borsky (1972) stated that exposure to repeat noise over a long period of time can uncomfortably disturb daily living and interfere with ongoing activity. Exposure to certain sounds has been found to cause stress and interfere with an individual's ability to sleep and work, particularly in activities that require concentration and high performance. This may

explain why individuals with chronic tinnitus find the perception of the tinnitus sounds annoying and how it influences their ability to attend to certain tasks and maintain a steady internal environment. Also, differences in sensitivity to the disturbing effects of tinnitus may reflect differences in one's ability to cope with stressful situations.

Rylander, (2004) emphasizes the fact that sounds not only have an influence on the body but also contribute to changes in the CNS (Rylander, 2004). These changes are not necessarily related to trauma or pain but rather involve a complex interaction between sound stimulus and physiological responses (Borsky, 1972). This suggests that a sound of certain intensity may stress the auditory system, activate HPA axis secretions, and disturb sleep.

Different techniques are used to measure the influence sound has on the activity of the HPA axis. One of the most promising techniques is to measure changes in the salivary glucocorticoids (e.g., cortisol levels) in stressed individuals exposed to noise. For example, Preussner et al. (1997, 1999) found that salivary cortisol levels increased after high levels of exposure to environmental noises greater than 60-70 dB. Waye et al. (2002) also reported that exposure to low frequency noises while working resulted in a decrease in saliva cortisol, particularly after several hours of work. This finding suggests that, similar to stress adaptation, individuals can adapt or habituate to certain sounds or noises of low frequency as long as their attention is shifted to a different task. To date, there is little data available to describe the physiological salivary changes associated with the exposure to bothersome sounds such as that of chronic tinnitus. These studies should have a high priority. The assessment of salivary hormonal markers of stress and the

ability to cope with stressful situations should be integral components of the assessment of the consequences of tinnitus.

Conclusion

An organism's ability to cope with chronic stress is regulated by the HPA and ANS and a network of behavioral responses. Stress can be indicated by the activity of the HPA axis and is most often revealed as an increase in cortisol and other stress related markers in blood plasma, saliva, and feces. There is an interchange between the neural activity of the HPA axis and the neural activity of cortical systems, which results in different emotional experiences in humans. A stressor can be presented in different forms; while some are physical, others can take the form of complex sounds. Bothersome sound, as in the case of tinnitus, can have a profound effect on the brain. Further studies are needed to determine how the brain either adapts to changes evoked by chronic tinnitus or whether this condition can lead to pathological changes in the auditory system.

Stress-Related Hormones

Hypothalamic Nuclei and Stress Related Hormones

In today's world, individuals encounter stress in different aspects of their lives. One way to adapt to stress is for the HPA axis to stimulate the secretions of a number of stress-related hormones. These responses are necessary for individuals to adapt to stressful situations. Failure to adapt can have an impact on the entire body and may lead to serious health conditions.

Hormones are messenger molecules that are released by specialized neurons in the brain and by the glands into the bloodstream. A hormone can have different

physiological and psychological functions. For example, stress hormones such as GCs not only influence the CNS and ANS reactions but can also influence auditory system functions. Exposure to varying degrees of stress alters the internal equilibrium state and, as a result, can trigger changes in the endocrine system. For example, reactions to stress are known to enhance secretions hormones such as glucocorticoids or cortisol, alpha amylase, melatonin, and neopterin in "cases of oxidative stress." Some of their effects are seen as changes in the mobilization of energy sources, while other changes require the individual to make adaptable alterations.

The focal function of the hypothalamus is to preserve a status of internal stability or homeostasis by producing chemicals that either stimulate or suppress hormone secretions from the pituitary gland (Turnbull & Rivier, 1999). The hypothalamus contains many nuclei. Since the wide range of function of these hypothalamic nuclei is not well defined (de Kloet, 1991), they are frequently studied as areas or regions. Table 1 shows a list of these hypothalamic nuclei regions and some of their related functions.
Although researchers have attempted to investigate these regions/nuclei and their related functions, further research is needed in order to assign specific functions to them. The hypothalamus is linked with the auditory system through the inferior colliculus (Adams, 1980). Although the extent of this connection is still unclear, specific hypothalamic nuclei have been associated with the regulation of different types of human hormones. For example, the sprachiasmatic nucleus (SCN) is associated with hormones that regulate circadian rhythm (Halasz, 2000, Levine, 2000) and expresses another wide range of hormone receptors (Jennes and Langub, 2000). Table 2 summarizes the four groups of

stress-related hormones and their functions. One hormone, melatonin, is related to the circadian cycle, whereas the other three hormones, cortisol, alpha-amylase, and neopterin, are related to stress and can exhibit both diurnal and circadian rhythms.

Table 1

Hypothalmic Nuclei Regions

Hypothalamic nuclei	Associated areas	Function
Preoptic region	Medial preoptic nucleus (MPO). Lateral preoptic nucleus. Lateral nucleus.	Copulation, drinking, and temperature. Feeding and sleep.
Anterior (supraoptic) region	Supraoptic nucleus (SON). Periventricular nucleus (PVN). Anterior nucleus (AN). Suprachiasmatic nucleus (SCN).	Vasopressin, oxytocin. Stress response, feeding, heat dissipation, parasympathetic responses, and circadian rhythms.
Middle (tuberal) region	Dorsomedial nucleus (DVN). Ventromedial nucleus (VMN). Arcuate nucleus (ARC). Lateral nucleus.	Inhibit feeding center; feeding, sleep, dopamine as prolactin inhibiting center.
Posterior (mammillary) region	Mammillary body. Posterior nucleus. Lateral nucleus.	Plays role in memory and attention, heat production, heat conservation, sympathetic responses, feeding and sleep.

Cortisol is the major hormone produced by the human adrenal cortex in response to stress. A prolonged chronic production of cortisol can lead to cell death and immunodeficiency. The effect of cortisol on the central nervous system has been examined in terms of its influence on sensory functions in rats' brains (de Kloet, 1991).

In humans, the influence of the GCs secretion of cortisol on the brain is examined mainly by considering four different areas of research described below.

Table 2

Stress Related Hormones

Hormone	Site of production	Hormone function
Circadian cycle melatonin	Produced by the pineal gland (small endocrine gland). The Suprachiasmatic nuclei SCN control the daily cycle.	Founds in the cochlea; multiple effects on the CNS anticonvulsant; enhancing GABA and benzodiazepine function. May play a role in improving tinnitus and protecting hearing.
Stress/ related hormones gcs: cortisol alpha-amylase neopterin	Hypothalamic-pituitary-adrenal axis (HPA). Corticotropin-releasing hormone (CRH), from the periventricular nucleus (PVN), adrenal cortex. Human monocytes/macrophages.	Abnormal basal and reactive cortisol levels in patients with tinnitus. Involvement is not fully investigated in tinnitus, yet; however, increased levels were found in relation to traffic noise exposure. Immune system and oxidative stress responses.

Cortisol

The first area of research focuses on studying the physiological changes observed in patients with either hypo or hypercortisolism. A number of investigators have found that behavioral changes in individuals with either hypo or hypercortisolism are possible to normalize with glucocorticoid therapy (Funder & Sheppard, 1987; Henkin, Greep, and Astwood, 1975).

The second area of research focuses on examining the changes in the circadian rhythm due to changes in cortisol secretions. Cortisol secretion is synchronized via a circadian rhythm. Its maximum level is in the early morning hours and its lower levels are during the first part of the night (Henkin, 1970). The synchronized nature of cortisol enables the study of hormonal changes in cortisol levels at different times of the day. Although the brain receptors of GCs are impacted by the circadian rhythm (de Kloet et al., 1991), there are circadian variances in receptor density and receptor occupancy in the brain (Folkard, 1979; Marshall & Don-chin, 1981). This suggests there are a number of limitations to the study of brain changes due to fluctuations in cortisol levels.

The third area of research examines the application of exogenous corticosteroids to normalize targeted hormone levels (Born et al., 1987a; Kopell et al., 1970). The findings of these studies disagree on whether enhanced cortisol levels result in inhibition or excitation of the central nervous system (Born et al., 1987b). For example, Fehm-Wolfsdorf et al. (1989, 1992) found a differential effect of the application of cortisone, specifically hydrocortisone and dexamethasone, on hearing and taste functions. The intake of hydrocortisone resulted in higher taste detection thresholds and auditory reflexes, while dexamethasone had a reverse effect on taste and auditory thresholds. The fourth and final line of investigation focuses on the study of the impact stress-induced cortisol secretions have on the CNS in comparison to non-stressful conditions.

Cortisol and tinnitus. One of the main complaints of individuals with chronic tinnitus is stress. Despite the clear indication of a link between chronic tinnitus and stress, this type of investigation has received little experimental attention. Hebert and Lupien

(2007) exposed eighteen participants with tinnitus (Mean age=68.8yrs, SD=5.7) and eighteen controls (Mean age=68.9, SD=5.5) without tinnitus using the Trier Social Stress Task (TSST). The researchers measured cortisol responses and feelings of stress and tinnitus intensity at different points of time up to one-hour posttest in order to measure cortisol levels at baseline and the participants responsiveness to stress and recovery time. The findings of this investigation suggest a dysfunctional HPA axis activity in the tinnitus group. The tinnitus group did not show an increase in cortisol level 20 minutes after exposure to the TSST test, but there was a significant group difference in cortisol levels at 30 minutes ($F(1,34)=11.2$, $P< .003$). These differences were related to a state of chronic stress in the tinnitus group. The findings of Hebert and Lupine's study were the first to provide physiological evidence that individuals with tinnitus exhibit delayed cortisol responses to an acute stress. This may also suggest that individuals with chronic tinnitus might have a delayed endocrine response to psychosocial stress.

Salivary Alpha Amylase (sAA)

The measurement of salivary biomarkers as indicators of stress has become available and popular during the past decade. While the measurement of salivary cortisol has been widely adapted and utilized in biomedical research (Hellhammerm Wust and Kudielka, 2009), other biomarkers, such as the salivary enzyme alpha-amylase, have only recently been looked at as stress-related reflections of sympathetic nervous system activity. In recent years, salivary measures including sAA have become increasingly important in investigating the psychological changes related to induced stress (Nater and Rohleder, 2009).

Salivary alpha-amylase can indicate autonomic activity (Nater and Rohleder, 2009). A number of studies have shown that alpha-amylase can be used to indicate autonomic nervous system activation in response to physical stressors. Gilman et al., (1997a) showed that sAA values were higher in response to physical stress (treadmill exercise), while Nexo et al. (1988) found higher values of sAA in relation to exposure to a high-pressure chamber. Others reported higher values after bicycle exercise (Chatterton et al., 1996) or exposure to cold water (Chatterton et al., 1996). Salivary alpha-amylase levels have also been considered in response to psychological stress (Bosch et al., 1996). Interestingly, while it may seem clear that sAA levels rise in response to physical stress, sAA responses to psychological stress seem to be more inconsistent.

Chatterton et al. (1997) examined changes in sAA levels in subjects preparing for skydiving. Their findings indicate a higher level of sAA prior to jumping, with the highest-level observed right after landing compared to sAA levels of those who did not jump.

Other investigators observed increases in sAA levels in response to psychologically stressful conditions, for instance in nonverbal communication in individuals with an intellectual disability (Yamaaguchi et al., 2006a), child health and social relationships (Granger et al., 2006), and in peer rejection paradigms in adolescents (Stroud et al., 2009). Although there are differences in the effect stress has on sAA, the majority of studies propose sAA changes in response to psychological stress, which suggests that levels of alpha-amylase can be used as a biomarker of stress-related bodily changes.

In conclusion, sAA increases when the autonomic nervous system activity is increased, and this can be used to show that increases in sAA values may represent changes in ANS activities. Although a number of studies have shown that sAA is sensitive to stress-related changes, the use of sAA as a stress marker is reasonably new. Further studies are needed to examine if sAA, like cortisol, can be considered a stress-related marker.

Salivary alpha amylase and tinnitus. Only recently has an interest in using sAA as a biological marker of stress been adapted and utilized for clinical use. To date, there are no studies that investigate the responsiveness of alpha-amylase to chronic tinnitus; however, a significant increase in sAA was found in relation to naturalistic traffic noise exposure. Wagner et al. (2010) examined twenty participants who were exposed to binaural traffic noise that contained noise levels of 75dB for 20 minutes. The researchers collected saliva samples for cortisol and alpha-amylase right after and before noise exposure. Their findings showed increased levels of sAA and cortisol concentration after noise exposure ($p=0.045$, $p=0.01$). The findings of this study suggest the probability of using sAA as a marker of stress in relation to induced noise.

Melatonin

Most of our regular endogenous cycles are controlled by rhythms responding to signals from the environment. Melatonin is an indoleamine, a compound that contains an amino group, synthesized from serotonin. Melatonin is produced by the pineal gland and secreted in a nocturnal pattern controlled by the endogenous circadian peacemaker (ECP). The circadian peacemaker is defined as a self-sustained rhythm generator that

synchronizes oscillators (Kriegsfeld, lance and Silver, 2006). Circadian rhythms are biological rhythms lasting approximately 24 hours that peak during the night (Arendt, 2006). Since melatonin secretions are regulated by a circadian rhythm, light exposure during the night or a dark phase rapidly inhibits melatonin secretions. Furthermore, the duration of the melatonin peak is inversely proportional to day length (Macchi et al., 2004).

Melatonin may also act as a signal to regulate reproduction and other physiological parameters in humans including sleep, immune function, and effective disorders (Macchi et al., 2004). Even though melatonin was discovered decades ago and has been linked to a number of conditions, the exact properties and functions of melatonin are not fully understood. After it is created, melatonin reaches almost all the body's tissue (Cardinali and Pevet, 1998) via blood diffused into capillaries and is most highly concentrated in the cerbero-spinal fluids (Tricoire et al., 2003). There is different melatonin binding sites in different parts of the nervous system and in peripheral organs. Furthermore, melatonin has a defined physiologic role, and it has been linked to sleep and mood disturbances (Pandi-Perumal et al., 2008). Future research should focus on investigating the impact melatonin has on other health conditions such as chronic tinnitus.

Melatonin and tinnitus. Sleep disturbances and insomnia are among the most common complaints of individuals with chronic tinnitus. Interestingly, the impact of melatonin on sleep in patients with tinnitus has been considered and investigated because the majority of individuals with chronic tinnitus have problems either staying or falling

asleep (Hallam, 1988). Melatonin has been proposed as a possible treatment for individuals with tinnitus and insomnia. Megwalu et al. (2006) investigated the effect of melatonin on tinnitus and sleep. Their study included 24 participants with tinnitus. Participants were instructed to take 3mg of melatonin a day for four weeks, followed by four weeks of observation. All tinnitus participants took the tinnitus handicap inventory (THI) and the Pittsburgh sleep quality index (PSQI). The finding of this study showed the mean score of the THI decreased significantly ($P<0.0001$) after melatonin intake. Furthermore, changes in the PSQI were associated with changes observed in the THI scores. Overall, their findings suggest that melatonin intake could improve tinnitus THI scores and sleep. Not surprisingly, the best improvement in sleep was observed in those with the worst sleep quality. Remarkably, there was no relationship between the observed changes and the severity of tinnitus.

Neopterin

Reaction to stress involves activation of both the endocrine and immune responses. Stress triggers the nervous system's active biomolecules such as cortisol. These biomolecules have been identified and related to stress adaptation and immune system reactions (Moynihan, 2003). They have also been used as stress markers. One of the markers produced by the immune system in relation to stress is neopterin, which occurs naturally in the body in oxidized form (Danova, 1998). Neopterin is a marker that reflects stages of immune system activation and oxidative stress (Fuchs et al., 1988).

Biological markers of oxidative stress such as neopterin have been examined in relation to a number of pathologies such as HIV (Fuchs et al., 1988), viral hepatitis

(Reibnegger et al., 1988), schizophrenia (Flatow, Buckley and Miller, 2013), acute rubella in children (Zaknun et al., 1993), and oxidative stress in noise-induced hearing loss (Henderson et al., 2006). Taken as a whole, these investigations are important in showing how oxidative chronic stress develops and activates inflammatory reactions and impacts different health conditions.

Neopterin and noise and tinnitus. Oxidative stress has been reported to impact inner hair cells resulting in cell death (Henderson et al., 2006). Heightened noise levels can lead to swelling and rupturing of the dendritic terminals of the auditory nerve afferent fibers—a process known as glutamate excitotoxicity (Pauel, Ruel Gervais, d'Aldin, et al., 1998). Excitotoxicity happens when the levels of glutamate in the synapses can over-stimulate the glutamate receptors on the postsynaptic cells, resulting in swelling of the postsynaptic cell bodies and dendrites (Kandel, Schawrtaz, & Jessel, 2000). Over time, the swelling might disappear; yet, the loss it causes in intermediate cells is permanent, leading to a shrinking of the size of the stria vascularies as a result of high levels of noise (Hirose and Liberman, 2003). Although noise is known to lead to hair cell damage and/or death, the extent of noise that might result in oxidative stress and how it triggers this cascade of events is not fully understood. Considering the nature and type of stress exhibited in conditions such as ringing in the ears will be of great benefit in examining the possibility of the role of the immune system in stimulating and modulating reactive inflammatory biomarkers in chronic tinnitus.

Conclusion

Stress-related hormones, such as those secreted from the adrenal and pineal gland, are found in the human auditory system. Accordingly, hormone distribution is not limited to the central nervous system (Jennes and Lngub, 2000). For example, GRs are found in both parts of the cochlea, the sensory organ of Corti, hair cells, and supporting cells, as well as the non-sensory spiral ligament and stria vascularies tissues. This indicates that stress-related hormones play a role in maintaining homeostasis and contribute to signal production in the inner ear. Future research should investigate the role of stress-related hormones and the specific underlying mechanisms and networks involved in the modulation of chronic tinnitus.

Tinnitus

Tinnitus Definitions and Prevalence

A sound that is perceived in the brain in the absence of an actual external acoustic stimulus is known as tinnitus (Jastreboff & Hazel, 1993). The exact underlying neural generators of this condition are unknown (Eggermont & Robert, 2004). Although almost all humans experience a temporary ringing in the ears at some point of their lives, when this phantom sound intensifies, becomes constant, and interferes with other daily life functions, it becomes problematic. The experience of tinnitus can be multifaceted. The complexity of this condition is due to the interaction of sensory and motor modalities in some cases and the cascade of associated emotional and psychological reactions associated with it.

Tinnitus is a subjective sound that each individual perceives differently which may explain the disparity in the ways tinnitus is studied. For example, one of the best known ways to study tinnitus is by quantifying its sensory features such as loudness, intensity, pitch, duration, location, onset, the individual's exposure to noise, the individual's exposure to ototoxic drugs, presence of hearing loss, and history of other neurological and health conditions. The difficulty with this way of studying tinnitus is that it lacks objectivity. Shargorodsky et al. (2010) reported that an estimated 50 million adults in the United States reported experiencing some form of tinnitus. Martines et al. (2010) indicated that the prevalence of tinnitus increases with age. The prevalence of tinnitus was found to be at 14.3% in the 60 to 69-year-old age range in the Shargorodsky et al. study. Overall, millions of people all around the world suffer from chronic tinnitus.

Studies have shown that for 15% of the individuals with chronic tinnitus, their experience is often associated with distress (Lewis et al., 1994). For many of them, their condition contributes to physical and psychological problems that adversely affect their ability to work and communicate and might reduce their overall quality of life. Chronic tinnitus can be an extremely handicapping and debilitating problem in adults, especially among the elderly. It is also a problem for young adults who are increasingly exposed to environmental noise.

Chronic tinnitus can be present in association with other medical conditions such as hyperacusis, which is sensitivity to sound, hearing loss, chronic headaches, depression, insomnia, stress, and anxiety (Halford & Anderson 1991; Folmer, Griest et al. 1999; Folmer & Griest 2000; Shargorodsky, Curhan et al. 2010). Furthermore, the

psychological effects of chronic tinnitus can result in adverse physical responses usually reported as elevated blood pressure, increased levels of stress cortisol, vomiting, and nausea (Hebert & Lupien, 2007; Henry, Dennis, & Schechter, 2005; Moller, 2000).

Different forms of tinnitus perception have been reported clinically. Tinnitus sounds are usually described as tonal, buzzing, hissing, or ringing. In most cases, these sensory features cannot be linked to or used to determine the etiology of tinnitus. Researchers believe that these qualitative features can be associated with a particular etiology. For example, tinnitus related to presbycusis, hearing loss due to aging, is described as resembling high-frequency cricket sounds. Tinnitus related to noise-induced hearing loss is characterized with hearing loss between 4 and 6 KHz and mostly resembles a ringing sound.

Another unique feature of tinnitus has been described in patients who can modulate the loudness, laterality, or tonality of their tinnitus using head and neck maneuvers or through the stimulation of certain head and neck areas. This phenomenon is known as “gaze-evoked-tinnitus” or somatic tinnitus and was first observed in patients who underwent surgery to remove large vestibular schwannomas. Remarkably, post surgery, these patients were able to modulate the intensity and loudness of their tinnitus by exaggerated eye movement (Levine et al., 2007)

Brozoski and Bauer (2005) suggested that following noise exposure, a reduction in the normal afferent input to the auditory brainstem occurs and may result in an inappropriate regulation of somatosensory inputs to the auditory system. These somatosensory inputs could partially replace the lost auditory inputs to become part of

the auditory stream and heard as sound. Because tinnitus can be perceived as different forms of sounds including ringing, music, buzzing, hissing, crickets, ocean roar, electrical wire tension, etc., it has been widely considered a pathology of the ear (Baguley, 2002; 2003).

The involvement of the morphological and physiological changes of the CNS, or any other system such as the ANS and the HPA axis with tinnitus, has somehow escaped attention. Only in recent years have researchers shifted their attention to consider the involvement of networks and systems other than the auditory system in the regulation and modulation of tinnitus perception and intensity.

Tinnitus Theories

Within the last few decades, one aim of tinnitus research was to identify the underlying neural mechanisms of tinnitus in order to develop a medical cure. Although many attempts have been made, there is no medical cure for tinnitus and its neural mechanisms have not yet been defined. Researchers agree tinnitus is a complex and probably one of the most misunderstood conditions. In many cases, tinnitus is associated with ontological conditions such as hearing loss. A greater effort has been placed on understanding how certain ear pathologies may lead to tinnitus; however, tinnitus can also be initiated by changes in the auditory central nervous system and can occur in the absence of other health or emotional conditions.

Evidence has been accumulating that shows other systems might also be involved in the generation of chronic tinnitus, such as the autonomic nervous system, the limbic system, and the central auditory nervous system (Eggermont, 2005; Eggermont &

Roberts, 2004; Heller & Bergman, 1935; Tyler & Baker, 1983); therefore, management and treatment plans should focus on investigating all possible networks involved in order to help individuals with chronic tinnitus.

Cochlear origin of tinnitus. Theories of cochlear origin indicate that damage to the cochlea, inner and outer hair cells, and the auditory nerve can result in tinnitus. Chery et al. (1994) suggested that the suppression and sensitivity of the inner hair cells to sounds are damaged when the outer hair cells connected to those inner hair cells are damaged. LePage (1995) reported that damage to the outer hair cells in the cochlea could cause an excitory drift in the sensitivity of the inner hair cells that might cause the phantom perception of sound. In support for this view, research has shown that cochlear hearing loss can cause tinnitus. Moreover, some patients with sensorinural hearing loss characterized by high frequency loss have tinnitus that corresponds in pitch to the frequency at which the hearing loss begins (Hazell, 1981).

In the 1995, Jastreboff refined this view when he suggested that the brain might try to overcome what it perceives as dysfunctional outer hair cells by increasing efferent neural activity to those outer hair cells. Without appropriate functioning outer hair cells, these efferent activities would change the central perception of the inner hair cells input and cause an inappropriate increase in activity, which may lead to the perception of tinnitus (Jastreboff, 1995). Furthermore, Baguley (2003) reported that when the inner hair cells are intact and the outer hair cells are damaged, a desynchronization between the tectorial and basilar membrane occurs. The tectorial membrane will press upon the

stereocilia of the inner hair cells, which causes the inner hair cells to depolarize leading to increased afferent activity.

The concept of the cochlear origin of tinnitus is often linked to exposure to loud noises or ototoxic drugs. It is believed that exposure to loud noises or ototoxic medications add to the creation of an "edge effect" by changing the neuronal tonotopical maps organization along the basilar membrane. In this area, normal neural activity regions are followed by regions of decreased neural activity influenced by lateral inhibitions that may cause the perception of chronic tinnitus. It has been proposed that the hearing pitch of patients with tinnitus matches the transition point from normal to reduced neural activity in the cochlea (Sahley, 2001; Tyler, 2000).

The concept of damaged inner and outer hair cells causing tinnitus has been examined in the work of Kaltenbach (2000). His findings showed that the administration of protective agents inhibited glutamate excitory effects on the auditory nerve fibers and worked as a protective agent against tinnitus. Additionally, it has been suggested that calcium plays a role in the transduction process and that changes in calcium concentration within the outer hair cells may play a role in the generation of tinnitus (Tyler, 2000). This view suggests that reduced intercellular and extracellular calcium concentration causes burst-firing behaviors in the auditory nervous system, consequently causing the perception of tinnitus.

Models that point to the cochlea or the auditory nerve as the sole generator of tinnitus have been long disproved by a number of researchers such as Baguley (2002, 2003) and Tyler (2000), who showed that tinnitus could be present after a complete

abolishing of the auditory nerve and the cochlea. These findings suggest that although tinnitus can be initially triggered by damage to the cochlea or the auditory nerve (Baguley, 2003; Jastreboff, 1990), the origin and perception of tinnitus is not purely peripheral.

Neural origin of tinnitus. A number of researchers utilize the concept of neural plasticity, a common concept in the field of audiology and hearing science, when implementing techniques and treatment options for individuals with hearing aids and cochlear implants. The concept of neural plasticity points out that even in the absence of sound, the brain is always active. Plasticity is a term used to describe long or short term changes in the brain's neural sensitivity as a result of alterations to synaptic inputs (Kaltenbach, Zhang, Finlayson, 2005). A neural concept of tinnitus suggests that there is always some type of background electrical activity in the auditory nerve, even in the absence of external stimuli.

Initial theories of neural generation of tinnitus were based on the hypothesis that tinnitus is caused by an increase in spontaneous neural activity (Evans et al., 1981). Evans et al. (1981) examined the cat cochlea after injections of salicylate at a dosage equivalent to that known to cause tinnitus in humans (400mg/kg). Their findings indicated the cats developed hearing loss and an increase in spontaneous activity in the auditory nerve. When a lower dosage of salicylate was used (200mg/kg), this effect was absent. Other investigations, such as that of Stypulkowski (1990), suggest that a blood vessel in close contact to the auditory nerve would induce an increase in neural activity that is damaging to the auditory nerve and, as a result, cause the sensation of tinnitus.

Moller (2000) also suggests that similar to phantom limb pain, tinnitus might result from changes to the central nervous system that lead to the reorganization of the brain's cortical maps. It has been proposed that although central reorganization begins at the molecular and cellular levels, it eventually leads to changes in the central auditory nervous system and causes the induction of tinnitus perception over time (Kaltenbach et al., 2005).

Eggermont (2005) describes tinnitus as a phantom auditory sensation experienced in the absence of external sounds. Most but not all cases are associated with hearing loss induced by age or noise exposure. This implies that tinnitus might be a condition generated in the brain after a hearing loss is present. Eggermont further indicates that downward regulation of intra-cortical inhibition induced by damage to the cochlea or the auditory pathways can lead to the perception of tinnitus.

Overall, Eggermont's views on tinnitus suggest that synchronization of the nerve fibers' neural activity can give rise to the perception of tinnitus (Eggermont & Roberts, 2004). This theory on tinnitus suggests that discontinuity in the low-level stimulus induced neural activity across auditory nerve fibers is caused by a functional loss of outer hair cells in regions where inner hair cells are undamaged. This will result in reduced spontaneous neural activity of nerve fibers in the hearing loss range and result in a reduction of lateral inhibition at more central levels. Reduced lateral inhibition of neurons with frequencies close to the "edge frequency" of hearing loss will result in hypersensitivity and hyperactivity in these neurons (Eggermont and Komiya, 2000). This view is based on a series of animal studies showing that the deprivation of input induced

by noise exposure or ototoxic drugs can lead to the reorganization of topographic maps of frequency-specific cells in the auditory cortex (Eggermont & Roberts, 2004).

Theories of neural plasticity and tinnitus suggest that the brain is capable of adapting to neural changes as a result of an exposure to sound of an unidentified source. These neural changes exhibit the ability to re-map the brain neural connections. Other researchers report that hyper-excitation of nerve synapses as a result of deprivation of input can cause an inactive nerve synapse in the auditory system to become an excitory nerve synapse. As a result, neural information can be redirected in the central nervous system and cause hyper-excitability or excessive neural activity throughout the auditory system (Lanting et al., 2008). Interestingly, excitation of nerve synapses can alter changes in the limbic system through their connection with the auditory system via the medial geniculate body (MGB) as well.

Moller (2006) suggests that excitation of nerve synapses can produce abnormal activation of what he refers to as the non-classical auditory pathway. The non-classical auditory pathway is linked to the limbic system through the MGB, the emotional center of the brain, and is suggested to be responsible for emotional distress associated with tinnitus. Furthermore, Moller (2006) suggests that this non-classical neural feedback loop can explain a number of psychological problems experienced by patients with tinnitus such as depression and anxiety, (Moller, 2006), auditory perception (Tyler and Baker, 1983), insomnia (Folmer et al., 1999), and sensitivity to loud noise (Eggermont, 2005). Research based on a central neural hypothesis of tinnitus focuses on the plastic changes of the brain resulting from inhibition and excitation within the central auditory pathways.

Tinnitus Distress: Characteristics and Networks

The experience of tinnitus varies from one individual to another. Variations in the experience of tinnitus are reported as changes in the level of intensity and severity of associated psychological distress factors among patients. The emotional impact of tinnitus has been of interest to researchers for a long time. Conditions such as annoyance, stress, depression, and insomnia have been found to interact with tinnitus. Additionally, a considerable proportion of individuals bothered by their tinnitus are also diagnosed with depression and anxiety disorders (Zoger et al., 2001). The question remains why some individuals are bothered by tinnitus while others are not and how different stressors act either alone or with others to cause tinnitus to become a chronic, bothersome condition.

Although the exact mechanism of tinnitus is not fully understood, the role of emotional, cognitive, behavioral, and psychological distress associated with this condition has been emphasized in current tinnitus models (Coles, 1984). The assessment of tinnitus has focused not only on quantifying the audiometric features of tinnitus, such as it is loudness and severity, but also to consider the negative impact it has on the psychological and social well-being of the individual. In summary, it is clear that people who show distress-related signs may be at risk of developing severe tinnitus in periods of demanding or traumatic life events. Further studies should investigate and bridge the link between a network of distress and tinnitus intensity and severity.

Tinnitus and Stress

Stress is known to exacerbate the severity and intensity of tinnitus perception in individuals with chronic tinnitus (Henry & Wilson, 2001; Nodar, 1996). Although the

exact pathways in which stress impacts tinnitus are not fully understood (Moler, 2006), research is beginning to explore the link between a stress network and tinnitus intensity network. Stress might modulate the intensity and strength of the tinnitus signal. Limited research has attempted to examine the biological markers of stress such as cortisol levels in individuals with tinnitus. For example, Hebert & Lupien (2007) investigated 18 subjects with tinnitus and 18 controls without tinnitus who were exposed to the Trier Social Stress Task. They measured their cortisol levels to investigate cortisol reactivity to psychosocial stress in tinnitus sufferers. Their findings indicate that there is direct psychological and physiological evidence of delayed and blunted responsiveness of the endocrine system to psychological stress in those with tinnitus.

While stress might have no effect on some individuals, it can contribute radically to the worsening of tinnitus intensity in others. Schmitt et al. (2000) report that stress could act as a potential trigger for sudden hearing loss and the onset of tinnitus. Moreover, Hallam et al. (1984) found that stress might adversely influence the habituation process, suggesting that it is possible for tinnitus symptoms to act as a stressor resulting in higher psychological arousal and psychological distress levels.

In summary, the brain is the master regulator of how we respond to different stressful situations over any period of time. Because chronic tinnitus is persistent in existence and can coexist with other conditions such as depression or insomnia, it can become overwhelming and alarming. Future research should focus on investigating the link between the physiological changes induced by stress and chronic tinnitus. The role

stress plays in the worsening of tinnitus intensity makes it necessary to establish a stress model for tinnitus.

Tinnitus and Anxiety

Tinnitus may directly impact an individual, by affecting their enjoyment of daily life activities, or indirectly by causing anxiety and sleep problems that interfere with daily life. Tyler and Baker (1983) mailed a postal survey to a tinnitus self-help group and found that 70% of 72 respondents included emotional problems in their list of common difficulties. Their investigation showed that a third of the respondents complained of tinnitus-related relaxation/irritation/annoyance and/or depression/despair/frustration problems. Johnson et al. (1996) found that tinnitus was associated with elevated anxiety traits and depression. Both anxiety traits (46.3 vs. 39.9, $p < 0.01$) and depressive tendency (14.4 vs. 7.1, $p < 0.001$) were significantly correlated with the overall tinnitus severity. Overall, the worse the tinnitus complaint, the greater the likelihood the individual will have a more anxious personality and a tendency to subclinical depression (Halford et al., 1991).

Anxiety is a common disorder in individuals with chronic tinnitus. A number of studies report anxiety ranges from 19% to 45% in individuals with chronic tinnitus (Belli et al., 2008). A recent investigation using an adapted version of the World Health Organization (WHO) short form of the composite diagnostic interview found twelve-month prevalence rates of 60% for generalized anxiety disorder, 83% for a specific phobia, 67% for a social phobia, 58% for agoraphobia, and 21% for panic disorder in a self-selected population of tinnitus patients (Anderson et al., 2004). The above

investigations show that anxiety is a common complaint among patients with tinnitus, which suggests an imbalance of neurotransmitters and possibly stress-related hormones. The impact anxiety has on worsening tinnitus intensity must be considered when investigating the underlying mechanisms of tinnitus.

Tinnitus and Sleep Deprivation (Insomnia)

Sleep disturbance, also known as insomnia, is a frequent complaint of individuals with tinnitus. According to Folmer and Griest (2000), the prevalence of sleep disturbance among tinnitus suffers ranges from 22% to 77%. Next to difficulties in hearing and stress, disturbed sleep is a common complaint (Hallam, 1988). Individuals with tinnitus usually report difficulty falling asleep or falling back to sleep because of the signal that is perceived as a bothersome sound such as ringing, hissing, buzzing, music, tension wire, crickets, etc.

Folmer and Griest (2000) use the term, "adjustment insomnia," to describe sleep disturbance brought on by tinnitus. Adjustment insomnia is the existence of an identifiable stressor such as tinnitus. The insomnia is expected to resolve as soon as the stressor is removed. The concept of adjustment insomnia can be true in cases of acute insomnia. On the other hand, if the insomnia is a chronic condition, then removing the stressor or tinnitus will have no influence on the insomnia.

According to Folmer and Griest (2000), insomnia occurs more often in recent-onset tinnitus. Forty-five per cent of individuals with tinnitus with onset of less than one year reported changes in their sleep pattern, while only 26% of those with tinnitus with onset of more than 11 years reported changes in their sleep. As for other studies, sleep

disturbance patterns were influenced by tinnitus loudness in some (Slater et al., 1983) but not in others (Meikle et al., 1984). Despite the fact that there is not yet an identified shared somatic mechanism between tinnitus and sleep, many individuals with tinnitus tend to believe that tinnitus causes a disturbed sleep pattern.

In many cases, insomnia and tinnitus are reinforced by one another. For example, individuals with tinnitus think of it as a sleep-preventing condition, which triggers a cycle of sleepiness in addition to mental and somatic hyper-arousal (Cronlrin et al., 2007). While attendance to the tinnitus sounds may weaken with time, it is the attitude and perception about tinnitus and how it affects sleep that encourages the persistence of the insomnia behaviors. Recent studies have shown that when insomnia and depression are associated with tinnitus, more often there is reduced tolerance and increased discomfort among the individuals with this condition (Alster et al., 1993).

Alster et al.(1993) assessed the reported prevalence and severity of sleep disturbance among those with chronic tinnitus. They investigated 80 military personnel without major psychiatric conditions who had tinnitus associated with noise induced hearing loss (NIHL). Their findings, consistent with other research, support the idea that self-rated severity of tinnitus was greater in those with a higher sleep-disturbance score. Scott et al. (1990) also reported that self-rated complaints about tinnitus focused on emotional and sleep disturbances as well as auditory perceptual difficulties.

Additionally, sleep disturbance and depression were found to be the psychosomatic factors that most strongly predicted the increased discomfort and decrease in tolerance of tinnitus. On the other hand, a number of studies have reported that sleep

disturbance is partially independent from other complaints such as emotional distress, suggesting that mood alone is unlikely to account for the presence of insomnia (Hallam, 1996; Hiller & Goebel, 1992). Other studies, however, have found a significant correlation between sleep disturbances and depression (Alster, Schemsh, & Ornan, 1993) and between sleep disturbances and tinnitus severity (Folmer & Griest, 2000). Clearly, insomnia is one of the risk factors for tinnitus (Holgers, Erlandsson, & Barrenas, 2000), which suggests that melatonin levels may be abnormal.

In summary, the type of information gathered about insomnia in individuals with tinnitus is usually limited to the questions in the tinnitus severity index and/or the tinnitus questionnaire survey, which makes it difficult for health-care professionals to fully recognize the challenges faced by individuals with tinnitus. Consequently, along with any self-reported improvement or worsening in individual sleep patterns, tracking different biological markers, such as changes in melatonin levels, is essential to improving the quality and type of tinnitus treatment and management plan.

Tinnitus Intensity and Annoyance

Over the last two decades, treatments for tinnitus have focused on the annoyance level reported by patients with tinnitus in the form of a scale ranging from one (less bothered) to ten (extremely bothered). Additionally, multifunctional connections between the auditory system and the limbic system have been looked at in grading the severity of tinnitus and associated psychological distress symptoms such as annoyance. Chronic distress is caused by tinnitus that is annoying; and in many cases, annoyance is the reason individuals seek professional help. Remarkably, Falkenberg et al. (2003) found that how

much individuals are annoyed by their tinnitus does not depend on the intensity or loudness of the tinnitus perception, but rather on the strength of the connection between the auditory system, the cerebral cortex, and the limbic and autonomic nervous system. This suggests that the strength of these connections is what differentiates annoyed tinnitus individuals from the non-annoyed.

Epidemiologic studies in Sweden and England similarly estimate that 14% to 18% of individuals with tinnitus complain of annoyance (Coles, 1984; Axelsson & Ringdahl, 1989); however, the degree to which individuals are annoyed by their tinnitus depends on a number of other factors. For example, tinnitus location (unilateral, bilateral, in the head), tinnitus loudness (mild, moderate, severe), tinnitus onset (sudden, gradual), tinnitus nature (consistent, intermittent) - all differ based on other medical and psychological factors. The degree to which individuals are annoyed by tinnitus is different as well, resulting in "rating annoyance" to be one of the most challenging tasks when assessing individuals with tinnitus.

Individuals rate how annoyed they are by their tinnitus by the intensity of the tinnitus signal (Tyler & Stouffer, 1989). For some, the sound of tinnitus is rated as very loud or louder than normal environmental noise, while for others, loudness does not interfere with other environmental noise and may not even be bothersome at all. The first group will have more difficulty habituating to tinnitus because they may perceive it as more damaging or intrusive.

This concept is very controversial. Some researchers, such as Meikle et al. (1984), have reported only a small or moderate correlation between tinnitus loudness and tinnitus

distress levels. The annoyance level is associated with the individual's perceived severity and the presence of other disabilities (Meikle et al., 1984). Although some individuals do not report any unpleasant emotional and behavioral consequences of tinnitus, others experience considerable distress including sleep disturbance, mood changes, anxiety, social withdrawal, depression, and annoyance. They may also develop serious mental disorders (Rizzardo et al., 1998; Langenbach et al., 2005).

Conclusion

In summary, there are many unanswered questions regarding the relationship between tinnitus and annoyance level. Not all variations in an individual's reaction to severe chronic tinnitus may depend on factors such as onset, duration, presence or absence of hyperacusis, and the presence or absence of other medical and mental conditions. A model that is not limited to identifying the auditory features of tinnitus, but also considers the physiological and psychological features of tinnitus, is needed to better identify different yet successful management plans. Nevertheless, if all cases of tinnitus were to be evoked by the same mechanism, it might be assumed that every individual with tinnitus would react to his or her tinnitus the same way, which is not true. In the studies conducted so far, this has not been the case, which suggests that there is more than one tinnitus mechanism. This position is supported by the observation that tinnitus appears to have many different causes*; therefore, the perception of tinnitus can be controlled but not cured*. To achieve a possible cure, it will be important to first identify all of the possible networks involved in tinnitus modulation and then eliminate any other unrelated abnormal activity that might not be the source of tinnitus perception. This

difficult task of inclusion and exclusion could be achieved by creating mechanism-based methods for tinnitus reduction.

Purpose of the Current Research

To date, there is no medical cure for tinnitus. Although different causes of tinnitus have been proposed, none of them is exclusive. While different theories have been proposed to identify the underlying characteristics of tinnitus, the exact physiological mechanisms—neurologic, peripheral sensory end organ or inner ear, and metabolic— are still unknown. Few or no studies have examined the levels of the four stress-related hormones, cortisol, alpha-amylase, melatonin, and neopterin, in individuals with chronic tinnitus. Additionally, few studies have looked at the differences in these hormone levels after an induced stress task in male participants with and without chronic tinnitus. The following research questions and hypothesis correspond to the aim of this study:

1. *Are baseline measures of cortisol, salivary alpha-amylase, melatonin, and neopterin greater in male subjects with chronic tinnitus?* It was hypothesized that male subjects with chronic tinnitus will exhibit a greater change in the four stress-related hormones governed by the hypothalamus. They would demonstrate a greater differential adrenal and/or autonomic nervous system response in reaction to a counting stress task than those changes in hormone levels in male subjects without tinnitus. The observed changes will be used as an indicator of the involvement of the hypothalamic pituitary adrenal axis in the modulation of the intensity and distress level of the tinnitus perception.

2. *Is reactivity to a stressor greater in male subjects with chronic tinnitus?* It was hypothesized that subjects with chronic tinnitus will exhibit a greater change in these four stress-related hormones in reaction to a backward-counting stress task, a stress-inducing condition, than any change in hormone levels in male subjects without tinnitus over four different time intervals - baseline, 5 min posttest, 30 min posttest, and 60 min posttest. Posttest testing of stress-related hormones in males with chronic tinnitus will reveal that males with tinnitus are more sensitive to the stressor when compared to those without tinnitus.
3. *Do physiological measurements at 5 min, 30 min and 60 min posttest differ from baseline?* It was hypothesized that both groups, with and without tinnitus, may exhibit some immediate difference between baseline and post-stressor responses, but the group with tinnitus will recover slower.

CHAPTER III

METHODS

Institutional Review Board (IRB) approval was obtained from the University of North Carolina at Greensboro (UNC-G) for this study. The study used regression analysis ANOVAs to assess the hypothesis that levels of the biomarkers will differ between groups Regression models were used to examine evidence of group differences on each of the four biomarkers, controlling for baseline measure, stress (PSS), sleep (PSQI), and tinnitus severity (TSI).

Participants

Twenty adult male subjects with no significant hearing loss, 10 with tinnitus and 10 without, between the ages of 18 and 35 years old, enrolled in this study. Subjects were classified into one of two groups: 1) subjects with tinnitus and no significant hearing loss (n=10) and 2) subjects without tinnitus and no significant hearing loss (n=10). Subjects were recruited by word of mouth and flyers distributed by the principle investigator.

All subjects had hearing thresholds within normal limits, between 1000Hz and 6000Hz bilaterally, and normal middle ear function. Subjects' audiometric thresholds and middle ear pressure were obtained before the saliva collection task took place. The subjects' health conditions were assessed using the medical intake history questionnaire (The UNC-G Medical History Questionnaire) that documents prior history of chronic

medical conditions such as seizures, brain injury, medication intake, and the presence of any other neurological disorder, such as multiple sclerosis, Parkinson's disease, etc.

Inclusion Criteria

All subjects included in this study were adult males between 18 and 35 years old whose hearing was within normal limits, i.e., between 1000Hz and 6000Hz bilaterally with thresholds of 30dBHL or less. They all had to display normal middle ear function, no diagnostic history of neurological disorders, and no history of medically identified conditions such as depression, anxiety, insomnia, or other psychiatric conditions.

Exclusion Criteria

Subjects were excluded from the study if they were younger than 18 or older than 35 years of age. Subjects who had any dental work within the 48 hours prior to sample collection were also excluded. Because many adults are exposed to some type of noise exposure through the use of headphones while listening to music, a mild notch at 4000 Hz and 6000Hz not exceeding 30 dB HL was accepted. Subjects were excluded if they had an air bone gap, which would indicate the presence of a conductive hearing loss. Furthermore, subjects were excluded if they were prescribed or currently taking any medications for depression, anxiety, stress, bipolar disorder, thyroid, schizophrenia, or insomnia.

Materials

Behavioral, tinnitus-specific, and general health data were obtained using the UNC-G Medical History Questionnaire (Appendices A), the UNC-G Tinnitus Intake History Questionnaire (THQ: Appendices B), the UNC-G Tinnitus Index Survey (TSI:

Appendices C), and the Perceived Stress Scale Questionnaire (PSS: Appendices D). In addition, information describing subjects' sleep and physical activity was obtained using the Pittsburgh Sleep Quality Index (PSQI: Appendices E) and the International Physical Activity Questionnaire (IPAQ: Appendices F).

Prior to their lab visit, the principle investigator phone-screened all subjects by phone to determine their enrollment eligibility for enrollment. Later, questionnaires were emailed to the eligible subjects, who were instructed to complete and bring them to their scheduled lab visit. Since some of the hormones collected observe a circadian rhythm, all visits were scheduled during the same time of the day – 6:00 pm. During their visits, audiometric, behavioral as well as physiological/stress-related biomarkers essays were collected.

Instrumentation and Equipment

Behavioral Testing: Questionnaires

Six questionnaires were administrated in this study. Participants without tinnitus only completed the medical history, perceived stress, PSQI, and IPAQ, while participants with tinnitus completed all six questionnaires. These questionnaires were:

1. The UNC-G Medical History Questionnaire, adapted from Meikle, Griest, & Press (1986) with permission from the Oregon Health Science University (11/10/94);
2. The UNC-G Tinnitus Clinic Medical History Intake Questionnaire (UNCG/TIHQ), adapted from Meikle, Gries,t & Press (1986) with permission from the Oregon Health Science University (11/10/94);

3. The UNC-G Tinnitus Index Survey, adapted from Meikle, Griest, & Press (1986) with permission from the Oregon Health Science University (11/10/94);
4. The Perceived Stress Scale Questionnaire, adapted with permission from the American Sociological Association, from Cohen, Kamarck, and Mermellstein (1983).
5. The Pittsburgh Sleep Quality Index (PSQI), adapted with permission from Buysse et al., (1989), University of Pittsburgh, School of Medicine (02/24/2014); and
6. The International Physical Activity Questionnaire (IPAQ), adapted with permission from Dr. Laurie Wideman, UNC-G Department of Kinesiology (02/23/2014).

Audiometric Testing: Hearing Screening

All subjects had to go through the same hearing tests - otoscope, tympanometry, and audiometer - in order to determine their hearing thresholds and middle-ear function. Subjects with tinnitus had to have their tinnitus pitch and loudness matches (LDL) measured.

1. Otoscope: A handheld otoscope (Welch Allyn) examination was performed on every subject prior to hearing testing. The subject's ear was gently pulled upward and backward. This type of movement moves the acoustic meatus in line with the ear canal. This test evaluates the external auditory canal and the

tympanic membrane, i.e., eardrum, and looks for any obstructions in the ear canal such as earwax.

2. Tympanometry: A Grayson Stadler Instruments (GSI) 33 admittance bridge was used for tympanometry, which provided information on middle ear function. A soft-tip probe is placed inside the ear canal to create a seal, and the GSI 33 then automatically measures middle ear pressure and compliance. (Calibrated 02/05/13).
3. GSI61 audiometer**:** The subjects' hearing thresholds, loudness discomfort levels, tinnitus pitch match, and tinnitus loudness levels were obtained using the GSI61 clinical audiometer. The GSI61 is a clinical audiometer developed with a two-channel design that allows for fast, accurate, pure tone and speech-testing using phones, TDH and insert, bone vibrator, and sound field speakers for output. It features both status and audiogram screen layouts for data presentation. It also has an articulating screen. (Calibrated 02/06/13).

Biological Biomarkers: Saliva Collection and Handling

1. Collection aid: The Salimetrics salivary collection aid (55mm x 12mm) was used to collect samples from all participants. Each saliva collection aid is individually wrapped in a foil pouch, good for one time use. All samples were collected using a passive drool technique, which is approved for use with any type of analysis.
2. Saliva collection vial: Salimetrics Cryovials were used. The 2mL Cryovials (10mm x 46mm) are freestanding, polypropylene vials with external threaded

caps for efficient collection of up to 2mL of whole, passive drool saliva. There is a white marking area to allow for easy sample identification; and they are designed to be stored in frozen (to -80C) conditions.

3. Futura silver under-counter freezer: All saliva samples were stored in a silver series compact manual freezer that is specifically designed for applications in the clinical and research field. This freezer has a temperature control capability that ranges from -15C degrees to -25C degrees and is preset to maintain -20C degrees at all times.

Procedures

Recruitment Procedure

Subjects were recruited through word of mouth, email, and flyers distributed by the principle investigator (PI). Prior to testing, the PI called and administered a phone screening survey to all participants in order to determine their enrollment eligibility. Upon completion, an email with an attached saliva collection instruction sheet was sent to those subjects who were selected to take part in the study. The subjects were instructed to avoid food, dental surgery, sugary drinks, alcohol, caffeine, nicotine, acidic drinks, and excessive napping or exercising on scheduled lab visits at least 60 minutes before testing. In addition, they were instructed not to brush their teeth within 45 minutes prior to sample collection in order to avoid any risk of lowering pH levels and influencing bacterial growth.

Upon arrival at the lab where the experiment's procedures were administrated, the consent form was explained to the subjects. A signed copy of the consent form was given

to the subject and a second copy was kept in the subject's de-identified file. All testing took place at the University of North Carolina at Greensboro's Neuro Lab located on the third floor of the Ferguson building. All paper copies of test forms, including the experiment consent forms, were securely kept in a locked file cabinet. All saliva samples were de-identified. Subjects were assigned a number that was used in the salvia testing and in subsequent computer statistical data sheets and data analysis. All of the stored saliva samples were marked and identified by the numbered code only; names were not associated with data or salvia samples. All information obtained in this study is strictly confidential unless law requires disclosure.

Saliva Collection Procedure

Four saliva samples from each subject were obtained and stored. Due to the sensitive nature of some of the targeted biomarkers and the influence of circadian rhythm on some of the targeted hormones, with peak time later in the day, all collection procedures took place the same time of day, beginning at 6:00 pm. Subjects were asked to sit in a large, comfortable, reclining chair on the patient side of a double-wall, soundproof booth. Four samples were collected at four different time intervals: baseline or pre-stress test, 5 minutes posttest, 30 minutes posttest, and 60 minutes posttest.

Induce Stress Procedure

Stress hormones such as cortisol and melatonin commonly exhibit a diurnal and circadian rhythms. Concentrations are the highest in the morning at the circadian peak, progressively decline during the day, and show rapid elevation after the first few hours of sleep. Care was taken to collect saliva samples at the same time of day in order to avoid

large individual differences in baseline concentrations. All saliva samples were collected at 6:00 p.m. Furthermore, in order to account for any differences in sAA, which reacts quickly and retrains to baseline within 20 minutes after a stressful challenge (unless the stress is very high in intensity), sAA assay were obtained in the 5 min posttest. They will probably be reflected at the 30 min posttest intervals as well. Cortisol and melatonin were assayed in the 30 and 60 min posttest. Figure 3 shows schematic illustrations of the study procedures.

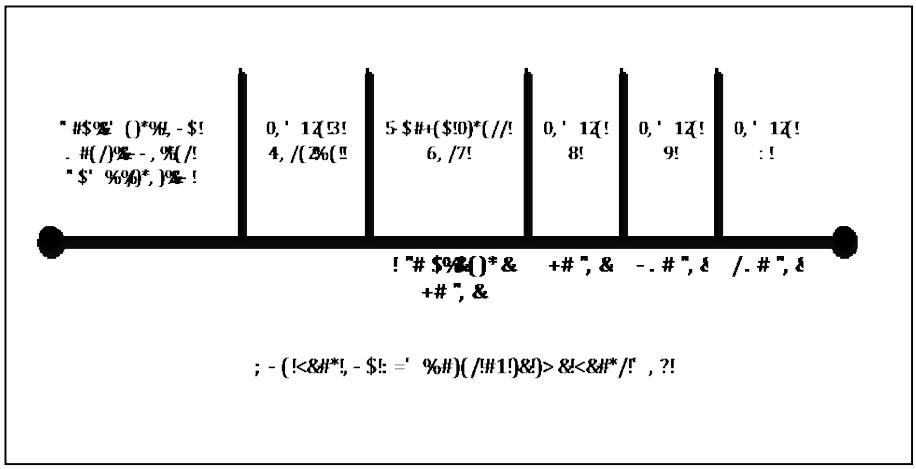

Figure 3. Illustration of the Study Procedures.

The Trier Social Stress Test is designed to induce stress in response to socially evaluative situations. Part of the TSST includes instructing subjects to count backwards from 1,022 in intervals of 13, which has proven to be effective in inducing physiological and hormonal changes in response to stress (Williams, Hagerty, and Brooks, 2004). The current study induced stress by asking subjects to count backwards an arithmetic mental task, starting at 5000 in intervals of sevens for up to five minutes. The PI remained inside the room with each of the subjects when administrating the backward-counting task to

ensure consistency; however, since the main purpose of the test is to induce stress, the PI did not help or correct subjects while they performed the task.

The PI used a timer that was turned on at the beginning of the task and set for exactly five minutes. The alarm would indicate the task was complete and it was time for the subject to stop. Upon completion of the counting task, the PI waited 5 minutes before collecting the first posttest sample, followed by the 30 min posttest and 60 min posttest samples.

Hormone Analyses Procedure at the Salimetrics Labs

The four saliva samples were obtained at baseline, 5 min posttest, 30m posttest, and 60m posttest from each test participant, marked with a de-identified code, packed in dry ice, and shipped to the Salimetrics lab for analysis of the stress-related hormones cortisol, alpha-amylase, melatonin, and neopterin. The Salimetrics Labs are an outside-certified laboratory specializing in the processing and analysis of salivary samples for a broad range of hormone assays as well as DNA markers via SNP and VNTR analysis. Further information and tables on the Salimetrics procedures can be reviewed in the supplementary methods (Appendices: G)

Statistical Designs and Analysis

Mean values of pre and post-stress levels for all four hormones were entered into an SPSS data spreadsheet along with each subject's demographic, audiometric, general and physical health, sleep, stress, and tinnitus data. Descriptive, inferential, and statistical tests, including parametric testing - regression ANOVAs models and a nonparametric version of ANOVA, the Kruskal-Wallis test -were computed.

Behavioral Data Analysis

One-way ANOVA F-tests along with descriptive data were computed using each of the IPAQ, PSS, PSQI, and TSI scores as dependent variables and the two groups, control vs. tinnitus, as the independent variables. Alpha level of .05 and 95% CI for all behavioral statistical were used. The following procedures were used to compute the scores of each of the questionnaires:

IPAQ. Scores of the International Physical Activity Questionnaire were calculated as the summation of the duration in minutes and the frequency in days of the activity. Subjects' scores were then classified into one of three main categories: inactive (category I), minimally active (category II), and health enhancing physical activity (category III).

PSS. The Perceived Stress Scale is a ten-item inventory designed to measure the perception of stress. Different daily live activities were presented and subjects were asked to indicate the level of stress experienced in each situation. To calculate the PSS scores of each subject, the obtained responses of four items on the stress scale (items # 4,5,7, & 8) were given a reverse value (e.g. 0=4, 1=3,2=2,3=1, & 4=0) and then added to the total response of each subject (items#1,2,3,6,9, & 10). Scores around 13 are considered average, while scores around 20 and higher corresponded to higher stress levels.

PSQI. The Pittsburgh Sleep Quality Index is an effective measure of sleep quality and pattern. It classifies sleep into either poor or good quality sleep. In order to do this, PSQI measures seven domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime

dysfunction over the last month. The subjects' scores to each of these seven domains were then calculated. Scoring is based on a 0 to 3 scale, whereby a score of 3 reflects the negative extreme on the Likert scale. A global sum of "5" or greater indicates poor sleep quality, while a global sum of less than "5" indicates good sleep quality.

TSI and THQ. Tinnitus related distress was assessed by the Tinnitus Severity History Questionnaire (THQ) and the Tinnitus Severity Index (TSI). The TSI questionnaire assesses the subjective psychological distress of tinnitus. It contains 12 items describing the impact of tinnitus on social and professional life, general well-being, concentration, leisure time, sleep, and overall emotional state (Meikle, Vernon, and Johnson 1984). Scores greater than 36 indicate bothersome tinnitus.

Physiological Data Analysis

The following statistical analyses were used to answer the following questions.

RQ 1: Are baseline measures of cortisol, salivary alpha-amylase, melatonin, and neopterin greater in male subjects with chronic tinnitus?

RQ 2: Is reactivity to a stressor greater in male subjects with chronic tinnitus?

Statistical Analysis

Assessment of overall group differences. First, to assess the hypothesis that levels of the biomarkers will differ for the tinnitus group, regression models were used to examine any evidence of group differences on each of the four biomarkers, controlling for baseline measure, stress (PSS), sleep (PSQI), and tinnitus severity (TSI).

Assessment of difference at each measurement point. Second, repeated measures and one-way ANOVAs were computed to test the physiological responses of

hormones for statistical significance across the four different time intervals of baseline, 5 min, 30 min, and 60 min posttest. The repeated-measures model was fitted using the four measures of reactivity as the within-subjects variable and the subjects group, control vs. tinnitus, as the between-subjects factor. We used an alpha level of .05 for the regression model statistical tests.

Nonparametric analysis. Kruskal-Wallis and one-way ANOVA Third, a nonparametric version of the one-way ANOVA test was computed between the four biomarkers using nonparametric analysis. Kruskal-Wallis measures used the four physiological responses of each biomarker across the different time intervals as the test variables, and the groups, control vs. tinnitus, as the grouping variables. Finally, a Bonferroni correction was made adjusting the p(sig) value by dividing the critical p(sig) value by the number of comparisons being made. The critical p(sig) =0; 05 value was divided by the total number of comparisons being made. The modified p(sig) values of (p=0.0125) were used in the interpretation of the nonparametric tests results. Then, the results of the nonparametric tests were used to "compare and contrast" with the one-way ANOVA test results.

RQ 3: Do physiological measurements at 5 min, 30 min and 60 min posttest differ from baseline?

Statistical analysis. First, differences from baseline were computed at each time interval by subtracting the mean value of the three physiological responses at the three time intervals from baseline measurement. A one-way ANOVA was then computed to test the physiological responses of hormones for statistical significance across the 5 min,

30 min, and 60 min posttest time intervals; and the results of the one-way ANOVA were then compared to the results of the Kruskal-Wallis test using the three physiological responses of each biomarker across the 5 min, 30 min and 60 min posttest time intervals as the test variables and the groups, control vs. tinnitus, as the grouping variables. In addition, a Bonferroni correction was made adjusting the p(sig) value by dividing the critical p(sig)=0.05 value by the number of comparisons being made; .05 value was divided by the total number of comparisons being made. The modified p(sig) values of (p=0.016) was used in the interpretation of the nonparametric tests results.

CHAPTER IV

RESULTS

General Descriptive and Demographic Data

Table 3 shows the general demographic data of age and pure-tone averages obtained from a total of 20 adult male subjects. The subjects' ages ranged from 18 to 31 years with a mean of =23.6 years, SD=4.47 for the control group (n=10) and a mean of 23.9 years, SD=3.78 in the tinnitus group (n=10). Additionally, a one-way ANOVA F-test revealed that the average age was not significantly different (F (1,18)=0. 026, and P= 0.873) between the two groups.

All subjects had normal hearing thresholds of 20 dB HL or less. Pure tone averages (PTA) for both groups were calculated for the right (control: M=19.66, SD=12.24; tinnitus: M= 16, SD= 10.72) and left ear (control: M=22, SD=9.93; tinnitus: M= 14.5, SD= 10.2). One-way ANOVA were also computed comparing the PTAs across the two groups. There was no statistical difference between the mean PTAs of the two groups for either the right or left ear (RE: F (1,18)=0. 508, p=0. 485; LE: F (1,18)=2.77, P=0. 113).

The second half of Table 3 also shows the mean values for tinnitus pitch (HZ) and tinnitus loudness (dB HL) as a function of the tinnitus group. The average pitch match for both left and right ears was approximately 3000Hz. The average pitch match for the right ear was in the mid-3000Hz (M=3525, SD=2180). Similarly, the average pitch match for

loudness was also calculated in dB HL for the right and left ear in the tinnitus group. Loudness match in the right ear varied from 13 to 44 dB HL (M=30.5, SD=11.24) while loudness match in the left ear varied from 13 to 58 dB HL (M=31.20, SD=13.33).

Table 3

General Demographics

Characteristic		Control	Tinnitus
Age	*M*	23.6	23.9
	SD	4.47	3.78
PTA/ right ear	*M*	19.66	16
	SD	12.24	10.72
PTA/left ear	*M*	22	14.5
	SD	9.93	10.2
Tinnitus pitch RE/LE (HZ)		Tinnitus RE	Tinnitus LE
	M	3525	3050
	SD	2180	1978
Tinnitus loudness RE/LE (dB HL)		Tinnitus RE	Tinnitus LE
	M	30.50	31.20
	SD	11.24	13.33

Table 4 shows the distribution of race among all 20 subjects. This study did not control for race, as race was not considered to be an exclusion factor.

A mean difference was observed among the control group (M= 2.9, SD=1.2), while the tinnitus group mean showed a normal distribution pattern (M=1, SD=0). As shown in table 5, the subjects ethnicity ranged from: white (1), African American (4), Parisian and African American*(1), Indian (3), and Asian American (1). All of the tinnitus group subjects belonged to the white ethnicity group.

Table 4

Subjects' Race/Ethnicity

Race	Control	Tinnitus
White American	1	10
African American	4	0
Parisian*	1	0
Indian	3	0
Asian American	1	0

Prior to testing, all subjects were instructed to refrain for at least an hour from certain dietary products that were high in protein, carbohydrates, sugar, and antioxidants. Additionally, the subjects were asked to complete a short dietary questionnaire inquiring about their eating habits. Table 5 shows the subjects' different dietary styles and classifies them according to high on protein/low on protein; high on carbohydrates/low on carbohydrates; and high on antioxidants/low on antioxidants.

Table 5

Subjects' Dietary Style

		# of subjects		Chi-square	
Dietary style	# of subjects	Control	Tinnitus	χ^2	*P* (sig)
High on protein	Yes	7	3	3.200	.074
Low on protein	Yes	3	4	0. 220	.639
High on carbohydrate	Yes	10	9	1.053	.305
High on antioxidant	Yes	0	3	3.520	.060
Low on antioxidant	Yes	10	7	8.571	.003*

Only one of the subjects in the tinnitus group reported a low carbohydrate diet. A chi-square test was used to test for differences between the different diet styles between the two examined groups. The results of the chi-square showed no significant relationship between the subjects group and their carbohydrate dietary style X^2 (1, N=20)= 1.053, P=. 305) Similarly, a chi-square test was performed, and no relationship was found between the subjects group and their high protein (X^2 (1, N=20)= 3.2, P=. 074) or high antioxidants (X^2 (1, N=20)= 3.52, P=. 060) dietary style. The results of a chi-square test did show a significant relationship between the subjects group and their low on antioxidant dietary style (X^2 (1, N=20)= 8.571, P=. 003, p<. 05).

Behavioral Data

Behavioral data was obtained on each subject using questionnaires with good psychometric properties that included the Tinnitus Severity History Questionnaire (THQ), the Tinnitus Severity Index (TSI), the Perceived Stress Scale (PSS), the Pittsburgh Sleep Quality Index (PSQI), and the International Physical Activity Questionnaire (IPAQ).

Tinnitus Related Distress (THQ and TSI) Questionnaires

Tinnitus-related distress was assessed by the Tinnitus Severity History Questionnaire (THQ) and the Tinnitus Severity Index (TSI). The THQ is an adaptation of a questionnaire developed at the Oregon Health Sciences University (OHSU; Portland, Oregon), from which we obtained permission to use in the clinic. It contains a set of 27 questions defining a number of subjective tinnitus characteristics such as loudness, onset, duration, location, tinnitus sound perceptions, discomfort level, and changes in daily

activity. The TSI questionnaire assesses subjective psychological distress brought on by tinnitus. It contains 12 items describing the impact of tinnitus on social and professional life, general well-being, concentration, leisure time, sleep, and overall emotional state (Meikle, Vernon, & Johnson 1984).

Table 6 shows the tinnitus group's tinnitus-specific characteristics of onset, duration, location, and self-report of tinnitus causality obtained from the THQ. Fifty percent (n=5) of the tinnitus subjects had a gradual onset of tinnitus (M=1.90, SD=. 738) lasting five or more years (M=3.30, SD=1.636). Forty percent (n=4) of the tinnitus subjects reported hearing tinnitus in both ears (M=2.50, SD=. 707). Both groups were equally divided in regard to what caused their tinnitus. Five subjects reported exposure to noise to be the primary cause of their tinnitus, and five-reported exposure to stressful events to be the primary cause of their tinnitus (M=1.5, SD=. 527).

Figure 4 illustrates the Tinnitus Severity Index (TSI). TSI is a 12-item questionnaire used to assess tinnitus distress. The TSI assesses the severity of a subject's tinnitus by inquiring if stress, anxiety, fatigue, or irritability exacerbates the severity of the tinnitus. Scores of 36 or higher are used to indicate bothersome tinnitus.

The higher the score, the more the subjects perceive their tinnitus to be a significant or debilitating problem.

The TSI scores had a mean of 19.20 and a SD=6.90, with a minimum score of 12 and a maximum score of 33 (<36). The responses obtained from this study's tinnitus subjects (n=10) indicate that tinnitus was not considered to be bothersome or deliberating (all scores < 36). Figure 5 illustrates the TSI scores of the tinnitus subjects (n=10).

Table 6

Tinnitus Subjects' Specific Characteristics from THQ

Tinnitus specific characteristic		Number of subjects (n=10)(%)	*M*	*SD*
Onset	Sudden	3 (30%)	1.90	.738
	Gradual	5 (50%)		
	I don't know	2(20%)		
Duration	< 1 year	1(10%)	3.30	1.636
	1-5 years	5(50%)		
	6+ years	4(40%)		
Location	Left ear	3(30%)	2.50	0.707
	Right ear	3(30%)		
	Both ears	4(40%)		
Illness associated with the onset of tinnitus	Noise Exposure	5(50%)	1.5	0.527
	Stress	5(50%)		

Tinnitus Severity Index

DIRECTIONS: For the questions below, please CIRCLE the number that best describes you

Does your tinnitus	Never	Rarely	Sometimes	Usually	Always
1. Make you feel irritable or nervous?	1	2	3	4	5
2. Make you feel tired or stressed?	1	2	3	4	5
3. Make it difficult for you to relax?	1	2	3	4	5
4. Make it uncomfortable to be in a quiet room?	1	2	3	4	5
5. Make it difficult to concentrate?	1	2	3	4	5
6. Make it harder to interact pleasantly with others?	1	2	3	4	5
7. Interfere with your *required* activities? (Work, home, care, or other responsibilities)	1	2	3	4	5
8. Interfere with your social activities or other things you do in your leisure time?	1	2	3	4	5
9. Interfere with your overall enjoyment of life?	1	2	3	4	5
10. Interfere with your ability to sleep?	1	2	3	4	5
11. How often do you have difficulty ignoring your tinnitus?	1	2	3	4	5
12. How often do you experience discomfort from tinnitus?	1	2	3	4	5

Figure 4. Tinnitus Severity Index. from Meikle & Griest, 1986. Copyright 1986 by Publisher.

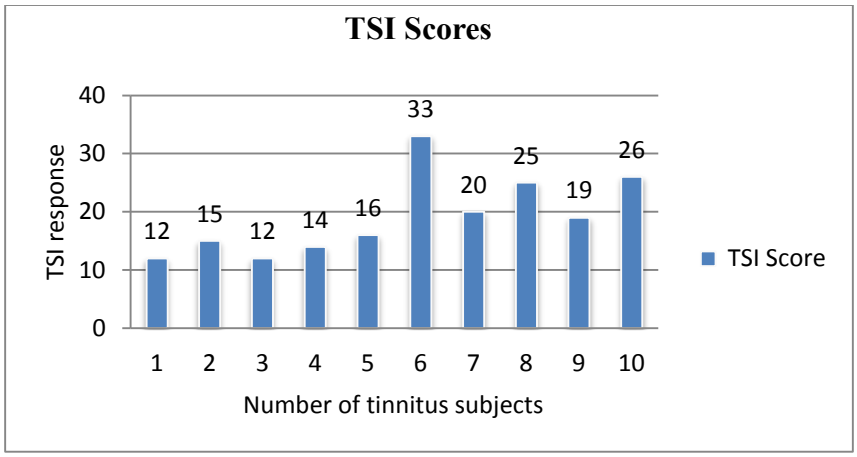

Figure 5. Tinnitus Severity Index (TSI) Scores.

The Perceived Stress Scale (PSS)

Prior to testing, all subjects were asked to rate their stress level on a scale from zero to ten, with zero indicating not stressed at all and ten feeling unbearably bad and out of control. Figure 6 shows the scale used to subjectively rate the subjects' stress levels. A one-way ANOVA was conducted to compare means among groups on their subjective rating of stress. The results of the one-way ANOVA F-test, as shown in table 7, indicate the mean ratings of stress were not statistically significant between the two groups.

Besides asking the subjects to rate how stressed they were before and after each of the sampling collection times, all subjects had to complete the Perceived Stress Scale (PSS) questionnaire, a tool that is designed to measure a subject's perception of stress. Very often, the PSS scores will correlate with the subjects' self-measurement of stress.

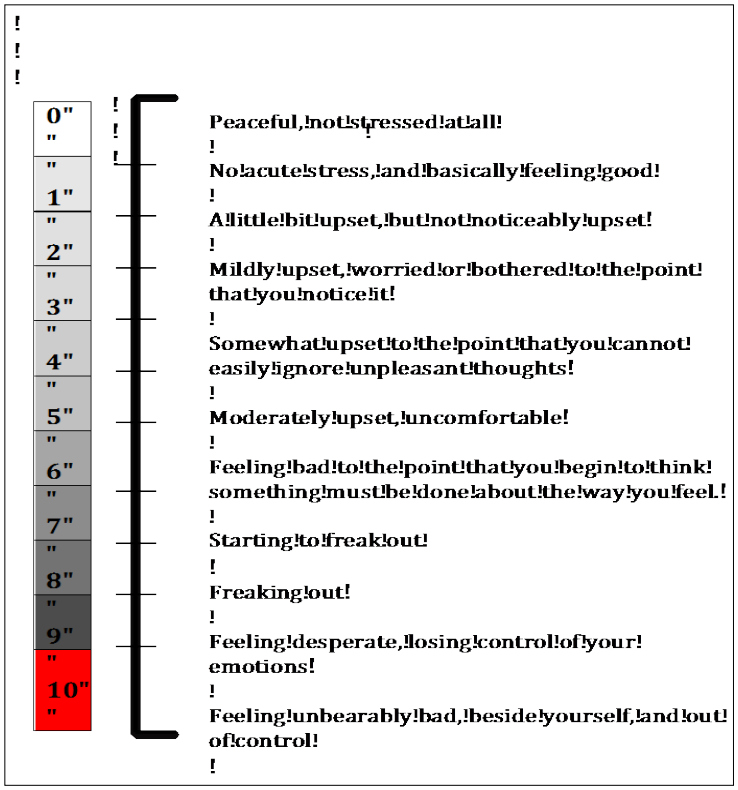

Figure 6. Subjective Stress Scales.

Table 7

Subjective Stress Scale Data

How stressed you are?	*M* *SD*	*F*-value *p*(sig)
Baseline	1.1 1.72	3.79 p=.067
5 min posttest	2.8 4.4	2.13 p=.161
30 min posttest	1 2.5	2.09 p=.165
60 min posttest	.90 1.8	1.38 p=.254

A Pearson's r correlation was computed to assess the relationship between the subjective scores of stress and the perceived stress scale scores. There was a positive correlation between the two variables ($r= 0.885$, $n=20$, $p<0.001$). Similarly, Spearman correlations were also computed to assess the relationship between the two variables. Overall, there was a positive correlation between the subjective rating of stress and scores obtained from the Perceived Stress Scale questionnaire ($r=0.0797$, $n=20$, $p< 0.001$). Table 8 shows the individual PSS scores of each subject.

Table 8

Subjects' PSS Scores

Control #	PSS scores	Tinnitus #	PSS scores
1	11	1	11
2	12	2	14
3	11	3	16
4	9	4	13
5	9	5	20
6	7	6	21
7	6	7	25
8	15	8	32
9	8	9	23
10	13	10	28

Overall, high PSS scores are found to be associated with greater vulnerability to stressful life-events. PSS scores were used to categorize subjects as either high-level or normal-level stress. Figure 7 illustrates the distribution of subjects in each of the two PSS stress categories.

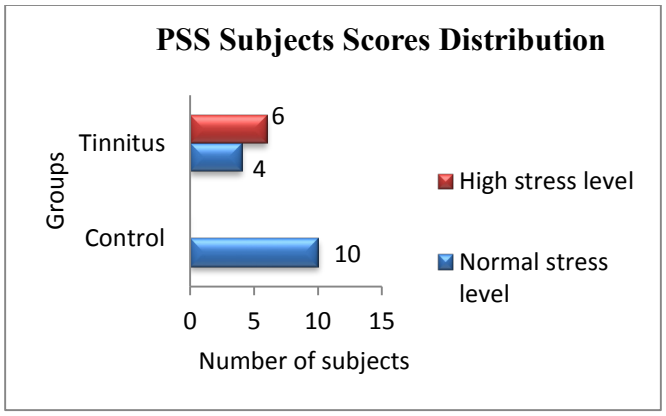

Figure 7. PSS Subjects' Scores Distribution.

Pittsburgh Sleep Quality Index (PSQI)

All the subjects of this study completed the Pittsburgh Sleep Quality Index questionnaire. The PSQI effectively measures sleep patterns and classifies sleep into either poor or good quality (Table 9). The PSQI contains 19 self-rated questions and 5 questions rated by the bed partner or roommate. To compute the PSQI scores, only self-rated questions were included in the scoring.

The 19 self-rated items were then combined to form seven component scores, each of which ranged from 0-3 points. A score of "0" indicated no difficulty, while a score of "3" indicated severe difficulty. These seven components were then added to record a global score with a range from 0-21 points. A total score of 5 or greater was used to indicate poor sleep quality, and a score of less than 5 was considered good sleep quality.

Means of the PSQI were (M=2.7, SD=1, 95% CI) for the control group and (M=4.9, SD=2.18, 95% CI) for the tinnitus group. A one-way ANOVA was conducted to

compare the means for the two groups. There was a significant difference (F (1,18)=6.818, p=0. 018).

Table 9

Subjects' PSQI Scores

Subjects number	PSQI global score	Sleep quality
	Control	
1	2	Good sleep quality
2	2	Good sleep quality
3	2	Good sleep quality
4	3	Good sleep quality
5	3	Good sleep quality
6	5	Poor sleep quality
7	2	Good sleep quality
8	2	Good sleep quality
9	4	Good sleep quality
10	2	Good sleep quality
	Tinnitus	
1	2	Good sleep quality
2	3	Good sleep quality
3	3	Good sleep quality
4	2	Good sleep quality
5	5	Poor sleep quality
6	7	Poor sleep quality
7	7	Poor sleep quality
8	7	Poor sleep quality
9	6	Poor sleep quality
10	7	Poor sleep quality

Out of the ten tinnitus subjects, six (60%) reported that tinnitus interfered with their ability to sleep, which indicates sleep disturbances were a mild problem for them, compared to only four subjects (40%) reporting that sleep disturbances were not problematic. Table 10 shows subjects PSQI scores.

A Pearson correlation was computed to assess the degree of linear relationship between the subjective individualized scores of sleep and the PSQI global scores. There was a significant positive linear correlation between the two variables ($r= 0.928$, $n=20$, $p<0.001$).

This study also assessed the relationship between the PSQI and PSS scores. A Pearson's r correlation was computed and found a positive linear relation between the PSQI and PSS scores ($r= 0.892$, $n=20$, $p<0.001$).

The International Physical Activity Questionnaire (IPAQ) Short Form

Both categorical and continuous data were obtained from the IPAQ short form. To categorize the subjects of this study, the summation of the volume in minutes and the number of days/sessions were computed. Table 10. The IPAQ short form subjects' scores category in minutes per day. The IPAQ suggests three different levels of physical activity to classify populations. All levels of physical activity take in consideration the total physical activity of all domains. The three proposed levels are:

- Category I: inactive,
- Category II: minimally active, and
- Category III: health enhancing physical activity (HEPA), i.e., highly active.

Table 11 shows the individualized scores of subjects in each of the two groups, as well as the type of activity and duration in minutes per day. The scores obtained from this study classified all of the control as well as the tinnitus group subjects as category II – minimally active. According to the IPAQ classifications, Category II or minimally active subjects can be further classified into one of three categories:

1. 3 or more days of vigorous activity of at least 20 minutes per day;
2. 5 or more days of moderate intensity activity or walking of at least 30 minutes per day; and
3. 5 or more days of combination of walking, moderate intensity, or vigorous intensity activities achieving a minimum of at least 600 min per week.

Table 10

Subjects' IPAQ Scores

#	Type of activity min/day	Category
	Control	
1	3 or more days of vigorous activity of at least 20minutes per day	II
2	5 or more days of walking of at least 30 minutes per day	II
3	5 or more days of walking of at least 30 minutes per day	II
4	5 or more days of walking of at least 30 minutes per day	II
5	3 or more days of vigorous activity of at least 20minutes per day	II
6	5 or more days of walking of at least 30 minutes per day	II
7	5 or more days of combination walking/moderate/vigorous act at least 600min/day	II
8	3 or more days of vigorous activity of at least 20minutes per day	II
9	5 or more days of walking of at least 30 minutes per day	II
10	3 or more days of vigorous activity of at least 20minutes per day	II
	Tinnitus	
1	5 or more days of walking of at least 30 minutes per day	II
2	5 or more days of walking of at least 30 minutes per day	II
3	5 or more days of walking of at least 30 minutes per day	II
4	3 or more days of vigorous activity of at least 20minutes per day	II
5	5 or more days of walking of at least 30 minutes per day	II
6	5 or more days of walking of at least 30 minutes per day	II
7	5 or more days of walking of at least 30 minutes per day	II
8	5 or more days of walking of at least 30 minutes per day	II
9	5 or more days of walking of at least 30 minutes per day	II
10	3 or more days of vigorous activity of at least 20minutes per day	II

The results of the one-way ANOVA showed that there was not a significant difference between the means of the two groups, as both groups had (M=2, SD=0).

Physiological Measure Analyses

Reactivity of all the four biomarkers, cortisol, salivary-alpha amylase, melatonin and neopterin, was computed using different reactivity measures including increment, ratio, percentage, and area under the curve (AUC). Tables 11, 19, 27 and 35 show the group of 20 subjects who were exposed to a stressor between baseline and 5 minutes. Saliva samples for cortisol, salivary alpha–amylase, melatonin, and neopterin assays were collected at baseline (0min), right after the stressor (5 min), and at 30 and 60 minutes after the end of the stressor. Incremental, ratio, and percentages were computed for each of the four biomarkers. Only AUCs were used as the dependent variable in the analysis of this study.

Cortisol Data

Assessment of Overall Group Differences

Table 11 shows the group of 20 subjects who were exposed to a stressor between baseline and 5 min. Saliva samples for cortisol assay were collected at baseline or 0min, right after the stressor at 5 min, and at 30 and 60 minutes after the end of the stressor.

To examine if different reactivity measures might influence changes in mean scores of the four physiological markers, a regression analysis model was fitted using the four measures of reactivity as the within-subjects dependent variable and the subjects group, control vs. tinnitus, as the between-subjects factor, after controlling for the effects

of sleep, stress, and baseline measures of cortisol biomarkers. Figure 8 illustrates the different reactivity measures for cortisol.

Table 11

Cortisol Reactivity

#	Increment	%	Ratio	AUC
		Control		
1	-.01	-52.17	2.09	.75
2	-.01	-14.61	1.17	4.28
3	-.03	-25	1.33	5.44
4	.00	-4.76	1.05	3.74
5	-.01	-21.74	1.28	1.94
6	-.03	-10.73	1.12	12.36
7	-.06	-45.65	1.84	6.16
8	.12	87.94	.53	12.51
9	-.02	-33.33	1.50	2.37
10	0.1	7.69	.93	6.16
		Tinnitus		
1	.02	0	0	1.02
2	-.02	-19.74	1.25	3.98
3	-.09	-25.07	1.33	18.83
4	.01	7.29	.93	5.91
5	.04	54.32	.65	6.10
6	.05	74.63	.57	6.57
7	-.11	-66.88	3.02	5.02
8	-.06	-75.29	4.05	2.71
9	.02	31.94	.76	4.74
10	.01	6.85	.94	5.66

The regression analysis results yielded no significant difference in cortisol ratio ($F(1, 15)=0.957$, $p=0.343$), cortisol increment ($F(1, 15)=.014$, $p=.909$), and cortisol percentage ($F(1,14)=.009$, $p=.926$) reactivity measures after controlling for the effects of sleep, stress, tinnitus severity, and cortisol baseline measure (0min). The same

regression model used the cortisol AUC as the dependent variable, with the tinnitus vs. control groups as the fixed variables. Similarly, the analysis revealed that cortisol AUCs of the between-subjects' tests yielded no significant difference (F (1,15)=. 089, p=. 883) after controlling for sleep, stress, tinnitus severity, and cortisol baseline measure. Although the regression analysis showed no statistical significance, the tinnitus group's cortisol AUC mean value (M=6.053, SD=4.79) was slightly higher than the control group's (M=5.573, SD= 4.03) mean value.

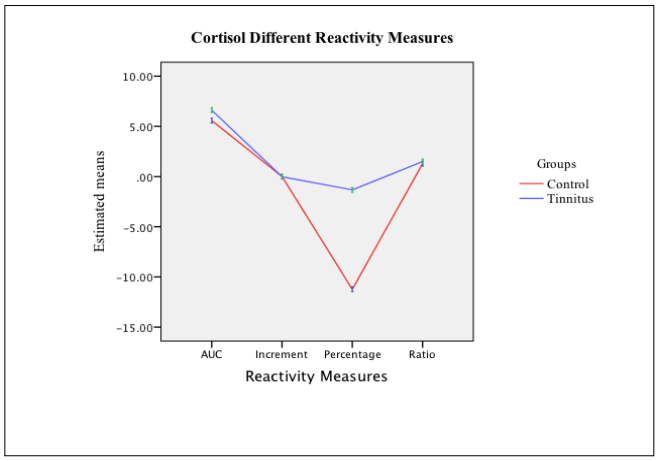

Figure 8. Cortisol Reactivity Measures.

Assessment of Differences at Each Measurement Point

In order to examine changes in the mean scores under the four different time points, repeated measures and a one-way ANOVA was computed using the four time points of baseline, 5 min, 30 min, and 60 min posttest as within-subjects variable and the control vs. tinnitus groups as the between-subjects factor. Figure 9 illustrates the mean cortisol levels across the different time intervals.

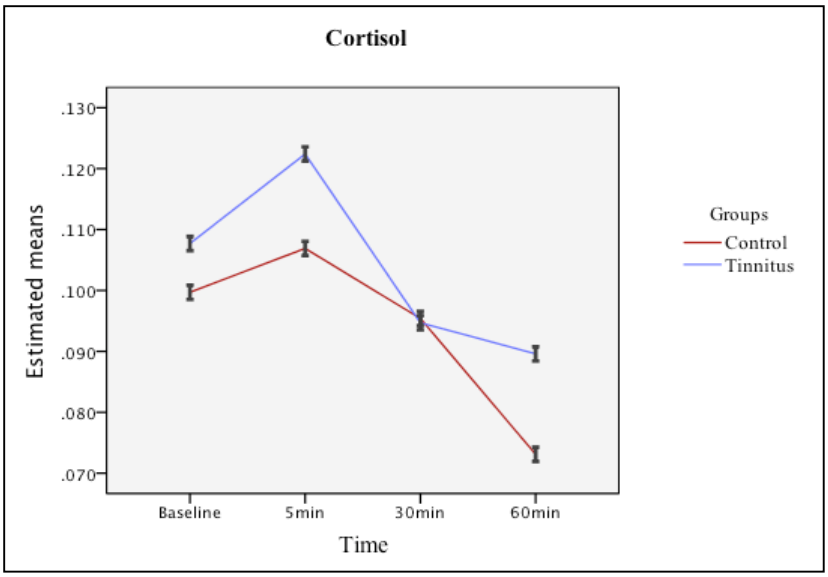

Figure 9. Cortisol Mean Values across Different Time Intervals.

With regard to the repeated-measures ANOVA conducted, the main effect of time was not statistically significant ($F(3, 54) = 2.759$, $p = .086$), while the main effect of participant group also failed to achieve statistical significance ($F(1, 18) = .086$, $p = .773$). Finally, the interaction between time and group membership on cortisol measurements was also not found to achieve statistical significance ($F(3, 54) = .222$, $p = .769$).

Table 12 shows the mean, standard deviation, and standard errors of the cortisol values at the different time points. The tinnitus group started with a slightly higher baseline mean (M=. 107, SD=. 098) when compared to the controls baseline (M=. 1, SD=. 060). Both groups' cortisol mean value increased after inducing stress, but the tinnitus group's increase was slightly higher (M=. 122, SD=. 112) compared to (M=. 106, SD=. 092) for the control group. Interestingly, at 30 minutes posttest, both groups tightly reached the same mean values of (M=. 094, SD=. 073) in the tinnitus group and (M=. 095, SD=. 079) in the control group; however, the tinnitus group's recovery was slower

post 30 minutes (M=. 089, SD=. 067) compared to the control group, which had a faster drop at 60 min posttest (M=. 073, SD=. 067). The results of one-way ANOVA F-tests yielded no significant difference across any of the baseline (F (1,18)=. 047, p=. 830), 5 min (F (1,18)= .113, p=. 740), 30 min (F (1,18)=. 00, p=. 984), and 60 min (F (1,18)=. 384, p=. 543) time measures.

Table 12

Cortisol at Different Time Intervals

Time		*M*	*SD*	*SE*
Baseline	Control	.099	.060	.019
	Tinnitus	.107	.098	.031
5 min	Control	.106	.092	.029
	Tinnitus	.122	.112	.035
30 min	Control	.095	.079	.025
	Tinnitus	.094	.073	.023
60 min	Control	.073	.050	.016
	Tinnitus	.089	.067	.021

Figures 10 and 11, illustrate the means across time points for all subjects, and Figure 12 shows a boxplot with outliers indicated. Two observations in the control group and one observation in the tinnitus group are identified as potential outliers. These observations might have been influencing the means of the two groups. These subjects were identified to be numbers 6 & 8 in the control group and number 13 in the tinnitus group. The previous analyses were repeated, omitting these three observations, and the conclusions remained unchanged. While the characteristics of these subjects were investigated, there was no good reason to exclude them. The only “noticeable” difference was in regard to the control subjects’ ethnicity. Both were African American.

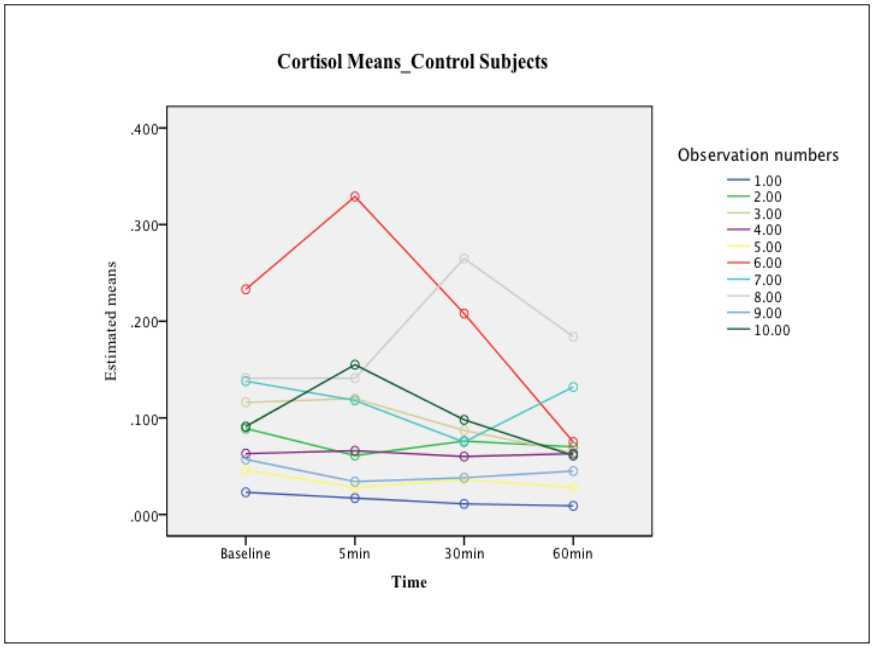

Figure 10. Control Cortisol Means across Time.

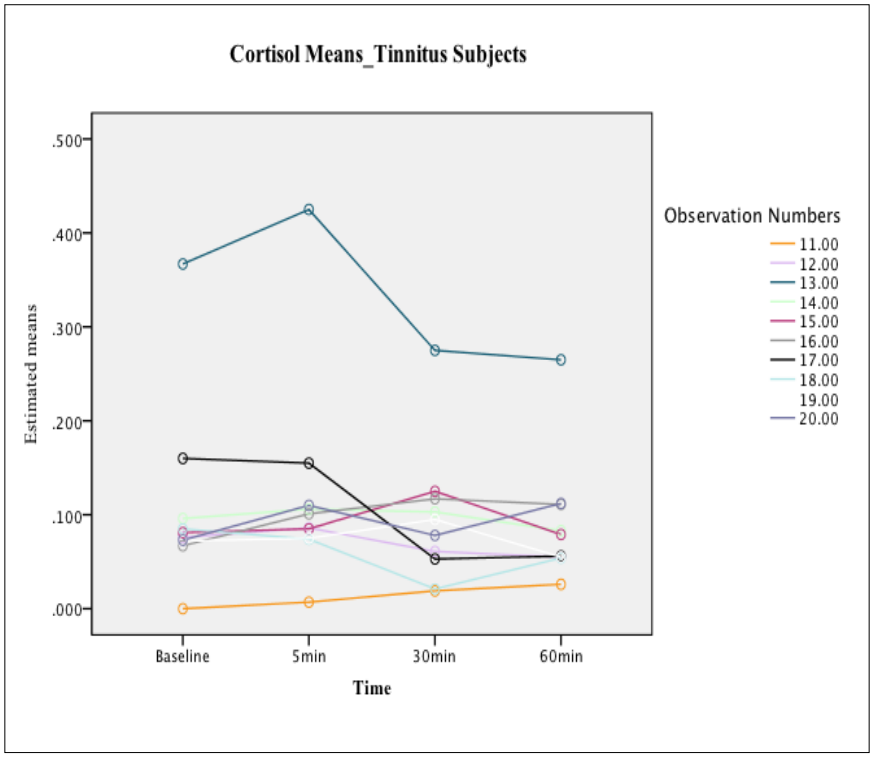

Figure 11. Tinnitus Cortisol Means Across Time.

In addition, since the regression model and ANOVA-tests were computed under the assumption that the distribution in the examined population is normal, residual plots were examined to check that assumption (figure 12). The pattern of the boxplot results (figure 13) of cortisol data does not indicate a serious departure from normality.

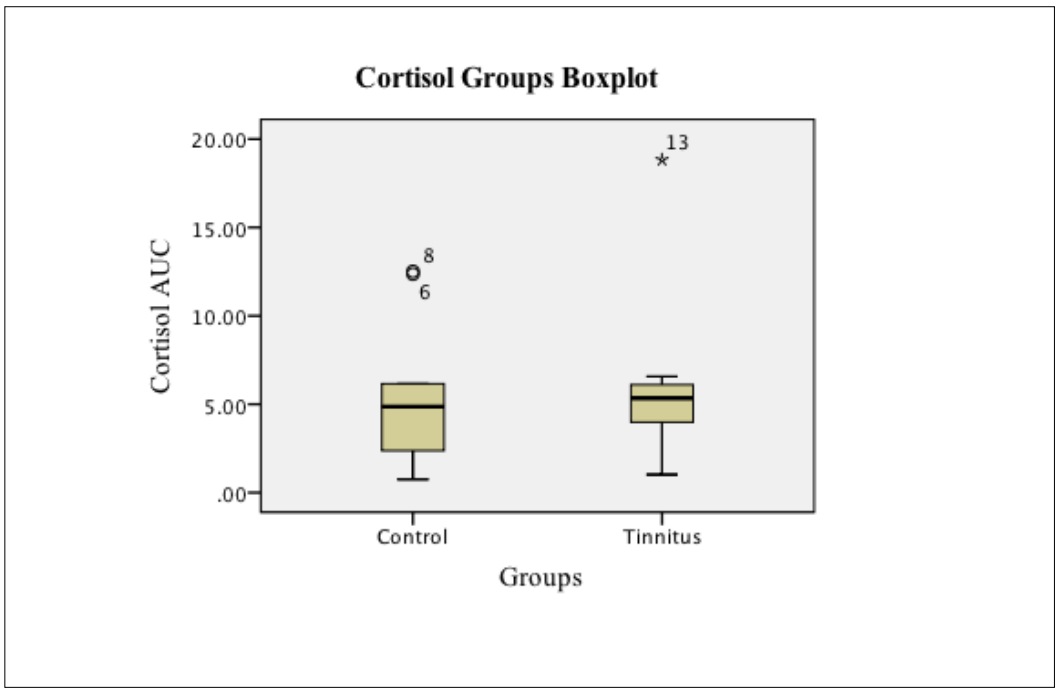

Figure 12. Cortisol Boxplot.

Kruskal-Wallis and One-Way ANOVA Test Results

Although serious departures from normality were not evident, nonparametric Kruskal-Wallis tests were computed to examine if samples originated from the same distribution. Table 13 and 14 show the results of the Kruskal-Wallis tests for cortisol. The nonparametric test revealed no statistically significant difference in the cortisol values across the different time intervals between the two groups, control vs. tinnitus.

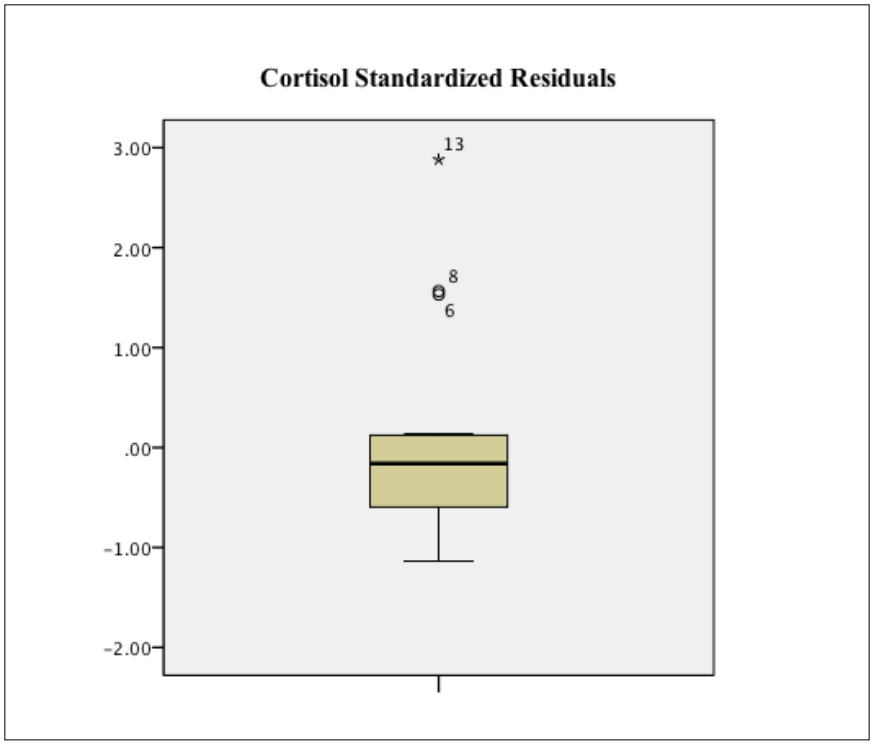

Figure 13. Cortisol Standardized Residuals Boxplot.

Table 13

Cortisol Mean Ranks

Time	Group	Mean rank
Baseline	Control	10.60
	Tinnitus	10.40
5 min	Control	10.15
	Tinnitus	10.85
30 min	Control	9.90
	Tinnitus	11.10
60 min	Control	10.00
	Tinnitus	11.00

Table 14

Cortisol Kruskal-Wallis Test Results

Cortisol	Baseline	5 min	30 min	60 min
Chi-Square	0.006	0.07	0.206	0.143
df	1	1	1	1
Asymp. Sig.	0.94	0.791	0.65	0.705

Note. Bonferroni correction applied: p (sig)=0.0125.

As observed in table 14, the p (sig) for the four different time intervals was insignificant and ranged from (x^2 =. 006, p=. 94) at baseline, (x^2 =. 07, p=. 791) at 5 min, (x^2 =. 206, p=. 65) at 30min, and (x^2 =. 143, p=. 705) at 60min posttest. The results of the Kruskal-Wallis tests were consistent with the ANOVA test results shown in table 15. The analysis of variance shows no significant effect of cortisol values at the different time intervals.

Table 15

Cortisol ANOVA Test Results

Cortisol	Baseline	5 min	30 min	60 min
F-value (1,18)	.047	.113	.000	.384
p (sig)	.830	.740	.984	.543

Cortisol Differences from Baseline

Table 16 shows the results of the subtracted cortisol mean values at 5 min, 30 min, and 60 min posttest from the baseline in both the control and tinnitus groups. The results show that overall; the tinnitus group had higher cortisol values than the control group.

Table 16

Cortisol Difference from Baseline

Time	Groups	*M*	*SD*	*SE*
5 min	Control	.0072	.040	.012
	Tinnitus	.014	.021	.006
30 min	Control	-.0043	.048	.015
	Tinnitus	-.0130	.055	.0176
60 min	Control	-.0266	.052	.0164
	Tinnitus	-.0224	.050	.0113

A one-way ANOVA F-test revealed no significant difference between cortisol measures at 5 min (F (1,18)=. 226, p=. 612, p<. 016), 30 min (F (1,18)=. 138, p=. 715, p<. 016), and 60 min (F (1,18)=. 135, p=. 718, p<. 016) posttest (table 17). Using the Bonferroni-adjusted critical p of .016. Furthermore, the results of the parametric one-way ANOVA were consistent with the results of the nonparametric Kruskal-Wallis test (table 18), which showed no significant difference at 5 min (x^2 =2.52, p=. 112, p<. 016), 30 min (x^2 =. 242, p=. 623, p<. 016), and 60 min posttest (x^2 =. 091, p=. 762, p<. 016).

Table 17

Cortisol Differences ANOVA Test Results

Cortisol	5 min	30 min	60 min
F-value (1,18)	.266	.138	.135
p (sig)	.612	.715	.718

Table 18

Cortisol Differences Kruskal-Wallis Test Results

Cortisol	5 min	30 min	60 min
Chi-Square	2.526	.242	.091
df	1	1	1
Asymp. Sig.	.112	.623	.762

Note. Bonferroni correction applied: p (sig)= .016.

Salivary Alpha-Amylase (sAA) Data

Assessment of Overall Group Differences

Saliva samples for sAA assay were collected at 0min baseline, right after the 5 min stressor, and at 30 and 60 minutes after the end of the stressor. The reactivity of sAA was computed similarly to that of cortisol by using different reactivity measures including increment, ratio, percentage, and area under the curve (AUC). Table 19 shows the group of 20 subjects who were exposed to a 5-minutes stressor between baseline and 5 min.

To examine if different reactivity measures might effect changes in mean scores of the four physiological markers, a regression analysis model was fitted using the four measures of reactivity as the within-subjects dependent variable and the subjects group, control vs. tinnitus, as the between-subjects factor, after controlling for the effects of sleep, stress, and baseline measure of the sAA biomarker. Figure 14 illustrates the salivary alpha-amylase different reactivity measures.

Table 19

Salivary Alpha-Amylase Reactivity

#	Increment	%	Ratio	AUC
		Control		
1	-38.70	-58.11	2.39	2487.75
2	29.80	65.76	.64	4608.50
3	-1.70	-4.05	1.04	3482.75
4	-78.70	-42.40	1.74	9115.75
5	9.20	14.31	.87	4027
6	86	51.62	.66	10349.50
7	-67.30	-39.15	1.64	5839.75
8	8.20	5.43	.95	8378
9	32.80	78.10	.56	3953
10	-21	-48.17	1.93	1453
		Tinnitus		
1	40	71.30	.58	4800.50
2	-23	-37.28	1.59	3359
3	10.50	21.47	.82	3596.25
4	-1.90	-9.36	1.10	1077.25
5	45.30	94.57	.51	4488.75
6	-12.80	-69.57	3.29	863
7	-31.50	-84.22	6.34	1136.25
8	-6.90	-47.92	1.92	684.75
9	.00	.00	1	1830
10	-56.70	-60.26	2.52	3266.25

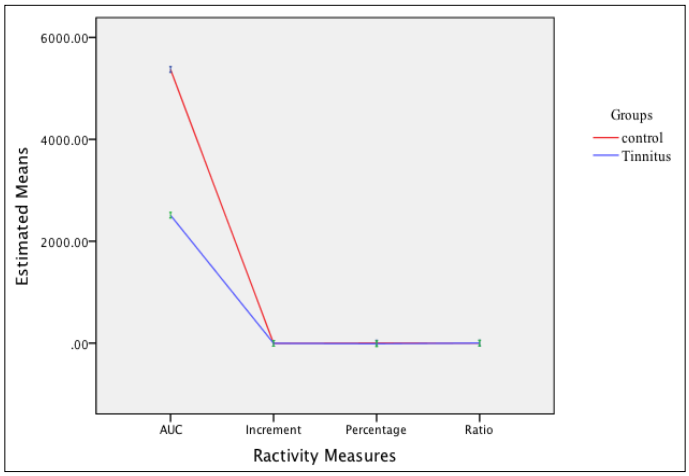

Figure 14. Salivary Alpha-Amylase Reactivity Measures.

The regression analysis results yielded no significant difference in sAA ratio (F (1, 15)=0.052, p=0.823), sAA increment (F (1, 15)=. 001, p=. 978), and sAA percentage (F (1,15)=. 018, p=. 896) reactivity measures after controlling for the effects of sleep, stress, tinnitus severity, and 0min *sAA* baseline measure. The same regression model used the sAA AUC as the dependent variable, with the tinnitus vs. control groups as the fixed variables. Similarly, the analysis revealed that cortisol AUCs of between-subjects' tests yielded no significant difference (F (1,15)=. 004, p=. 949) after controlling for sleep, stress, tinnitus severity, and sAA baseline measure. Although the regression analysis showed no statistical significance, the tinnitus group's sAA AUC mean value (M=2510.20, SD=1566.34) was significantly lower than the control group's (M=5369.55, SD=2975.72) mean value.

Assessment of Differences at Each Measurement Point

To examine changes in the mean scores under the four different time points, repeated measures and a one-way ANOVA were computed using the four time points, baseline, 5 min, 30 min, and 60 min posttest, as the within-subjects variable and the control vs. tinnitus subjects groups as the between-subjects factor. Figure 15 illustrates mean sAA values across the different time intervals.

Table 20 shows mean, standard deviation, and standard errors of the sAA values across the different time intervals. These results show that the sAA pattern of reaction was quite distinctive for both groups. As illustrated in figure 15 and table 20, the control group exhibited higher levels of sAA at baseline (M=98.60) compared to (M=42.84) in the tinnitus group. Furthermore, after introducing the stressor, the control group

responded with a decrease in sAA (M=80.55) values, while the tinnitus group exhibited the opposite pattern of reaction as an increase in sAA values (M=48.24).

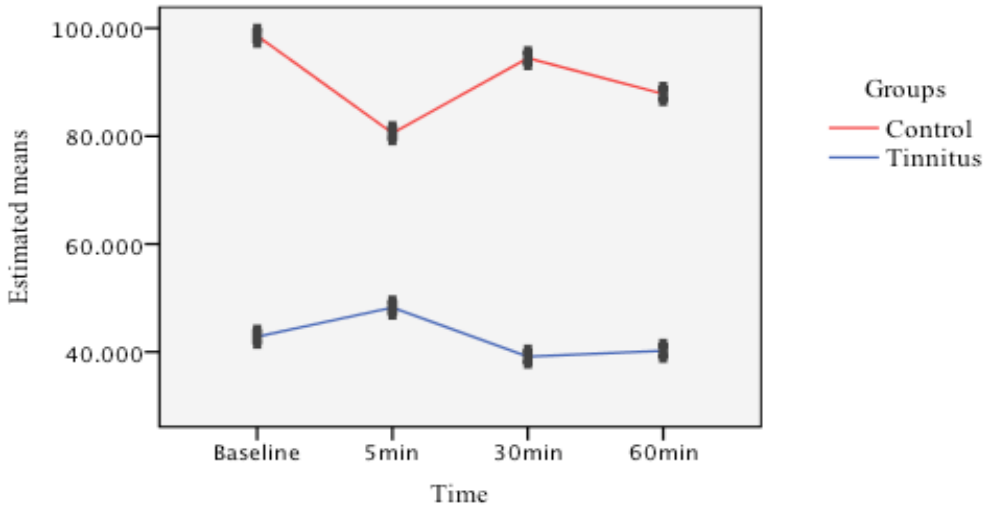

Figure 15. Salivary Alpha-Amylase Mean Values across Different Time Intervals.

Table 20

Salivary Alpha-Amylase at Different Time Intervals

Time		*M*	*SD*	*SE*
Baseline	Control	98.60	61.51	19.45
	Tinnitus	42.84	24.38	7.71
5 min	Control	80.55	54.86	17.34
	Tinnitus	48.24	28.16	8.90
30 min	Control	94.46	69.07	21.84
	Tinnitus	39.14	33.89	10.71
60 min	Control	87.81	48.04	15.19
	Tinnitus	40.21	30.06	9.50

In the repeated-measures ANOVA conducted, the main effect of time was not found to achieve statistical significance ($F (3, 54) = .205$, $p = .847$), while the main effect of participant group was found to achieve statistical significance ($F (1, 18) = 7.754$, $p = .012$). Finally, the interaction between time and group membership was not found to achieve statistical significance ($F (3, 54) = .648$, $p = .551$).

Correspondingly, the same pattern of reaction was also observed at 30 min posttest, with the tinnitus group seeming to follow the opposite reaction from that of the controls. While the sAA values increase in the control group at 30 min posttest ($M=94.46$), the sAA values for the tinnitus group decreased ($M=39.14$) at 30 min posttest ($M=39.14$, $SD=33.89$) and at 60 min posttest ($M=40.21$, $SD=20.06$). Additionally, the results of the one-way ANOVA F-tests revealed that there was a statistical significance at baseline ($F (1,18)= 7.1$, $p=. 016$, $p< .05$), 30 min ($F (1,18)= 5.16$, $p=. 035$, $p<. 05$), and 60 min ($F (1,18)=7.05$, $p=. 016$, $p< .05$) posttest, but no significance was observed at 5 min posttest ($F (1,18)= 2.74$, $p=. 115$, $p< .05$).

Alpha-amylase boxplot and standardized residuals were computed and showed no presence of outliers for sAA in any of the two groups, control vs. tinnitus (Figures 16 & 17). The regression model and the one-way ANOVA were computed under the assumption that the distribution in the population examined is normal. To check that assumption, residual plots were examined. The pattern observed in figures 16 and 17 of sAA data does not indicate a serious departure from normality.

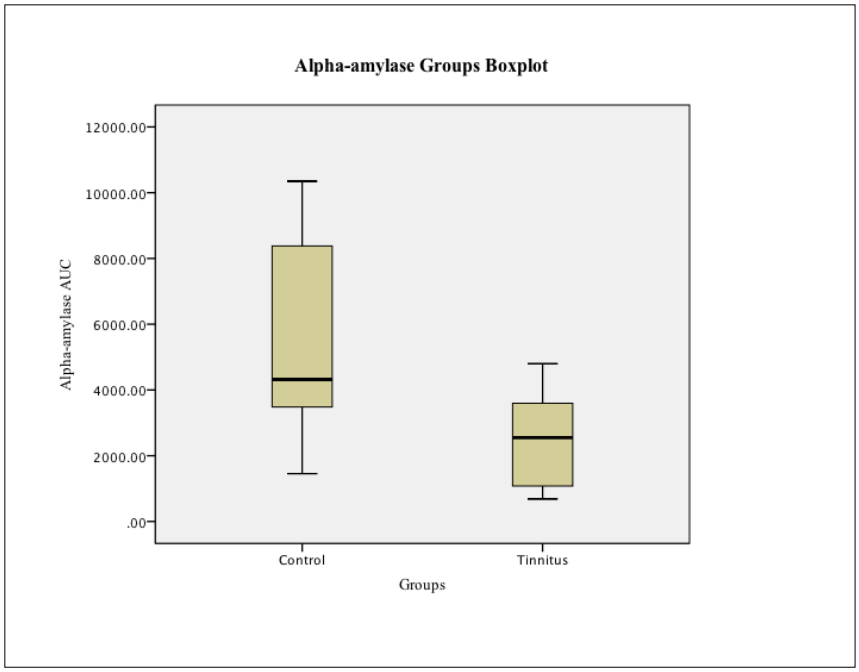

Figure 16. Salivary Alpha-Amylase Boxplot.

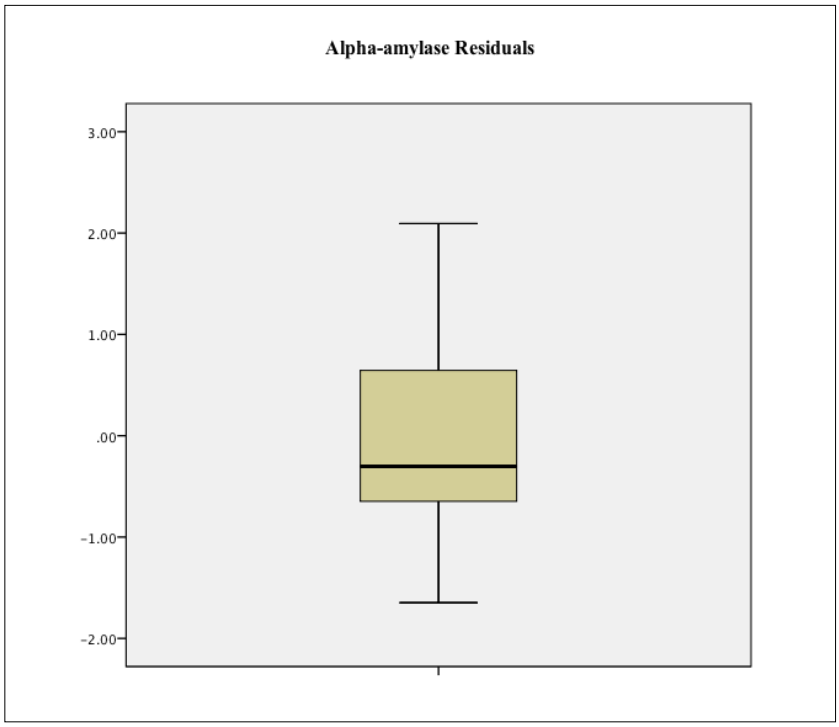

Figure 17. Salivary Alpha-Amylase Standardized Residuals Boxplot.

Kruskal-Wallis and One-Way ANOVA Tests Results

Tables 21 and 22 show the results of a nonparametric Kruskal-Wallis test for sAA. A Bonferroni correction was applied to the p(sig) level, and the modified p(sig) level was set at $p<0.0125$.

The nonparametric test revealed no statistically significant difference in the sAA values at baseline (x^2 =5.147, p=. 023), 5 min ((x^2 =3.296, p=. 069), and 30 min posttest (x^2 =4.80, p=. 028); however, a significant difference was observed at 60 min posttest (x^2 =7, p=. 008). As shown in tables 23 and 24, none of the ANOVA results were statistically significant at the 0.0125 levels.

The only observed discrepancy was at 60 min posttest where the Kruskal-Wallis p(sig) value was 0.016 and the ANOVA p(sig) was 0.008. Both p-values were almost the same, however, and cannot be used to fully draw a conclusion regarding differences in sAA.

Table 21

Salivary Alpha-Amylase Mean Ranks

Time	Group	Mean rank
Baseline	Control	13.50
	Tinnitus	7.50
5 min	Control	12.90
	Tinnitus	8.10
30 min	Control	13.40
	Tinnitus	7.60
60 min	Control	14.00
	Tinnitus	7.00

Table 22

Salivary Alpha-Amylase Kruskal-Wallis Test Results

Alpha-amylase	Baseline	5 min	30 min	60 min
Chi-Square	5.147	3.296	4.806	7.000
df	1	1	1	1
Asymp. Sig.	.023	.069	.028	.008

Note. Bonferroni correction applied: p (sig)=0.0125.

Table 23

Salivary Alpha-Amylase ANOVA Test Results

Alpha-amylase	Baseline	5 min	30 min	60 min
F-value (1,18)	7.1	2.74	5.16	7.05
p (sig)	.016	.115	.035	.016

sAA Differences from Baseline

Table 24 shows the results of subtracted sAA mean values at 5 min, 30 min, and 60 min posttest from baseline in both groups, control vs. tinnitus. The results show that the tinnitus group exhibited a higher decrease in sAA values when compared to the control group after 5 min posttest (M= -3.70, SD=30.87) when compared to the controls (M= -4.14, SD= 49.44).

A one-way ANOVA F-test revealed no significant difference between cortisol measures at 5 min (F (1,18)=3.12, p= .094, p<. 016), 30 min (F (1,18)=. 001, p=. 981, p<. 016), and 60 min (F (1,18)=. 201, p=. 659, p<. 016) posttest. Table 25 shows alpha-amylase differences from baseline parametric ANOVA test results.

Table 24

Salivary Alpha-Amylase Differences from Baseline

Time	Groups	*M*	*SD*	*SE*
5 min	Control	-18.05	41.32	13.06
	Tinnitus	5.40	7.16	2.26
30 min	Control	-4.14	49.44	15.63
	Tinnitus	-3.70	30.87	9.76
60 min	Control	-10.79	49.28	15.58
	Tinnitus	-2.63	29.72	9.39

Table 25

Salivary Alpha-Amylase ANOVA Test Results

Alpha-amylase	5 min	30 min	60 min
F-value (1,18)	3.12	.001	0.201
p (sig)	.094	.981	.659

Furthermore, the results of the parametric one-way ANOVA were consistent with the results of the nonparametric Kruskal-Wallis test (tables 26 & 27), which showed no significant difference between the 5 min (x^2 =2.987, p=. 089, p<. 016), 30 min (x^2 =. 000, p=1, p<. 016), and 60 min posttest (x^2 =. 023, p=. 880, p<. 016) time measurements.

Table 26

Salivary Alpha-Amylase Differences Kruskal-Wallis Test Results

Alpha-amylase	5 min	30 min	60 min
Chi-Square	2.987	.000	.023
df	1	1	1
Asymp. Sig.	.089	1.00	.880

Note. Bonferroni correction applied: p (sig)= .016.

Melatonin Data

Assessment of Overall Group Differences

Table 27 shows the group of 20 subjects who were exposed to a 5-minute stressor between baseline and 5 min. Saliva samples for melatonin assay were collected at 0min baseline, right after the 5 min stressor, and at 30 and 60 minutes after the end of the stressor. Changes in mean scores under the four different reactivity measures of increment, ratio, percentage, and AUC were tested using a regression analysis model that was fitted to the four measures of reactivity as the within-subjects dependent variable and the subjects groups, control vs. tinnitus, as the between-subjects factor, after controlling for the effects of sleep, stress, and a baseline measure of any melatonin biomarker.

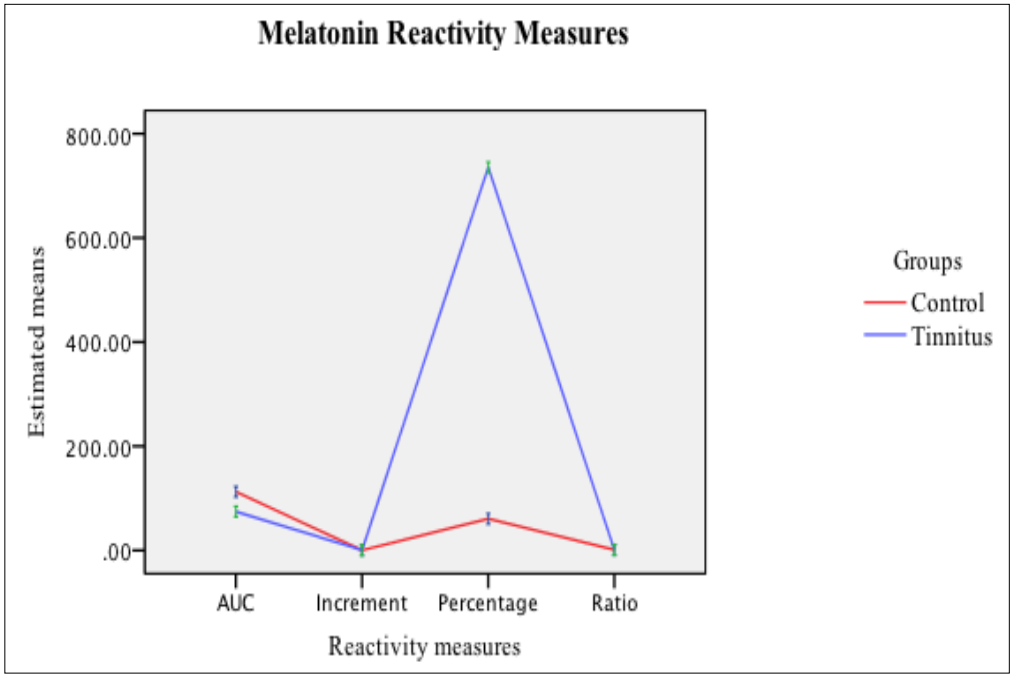

Figure 18. Melatonin Reactivity Measures.

Table 27

Melatonin Reactivity

#	Increment	%	Ratio	AUC
		Control		
1	.15	21.74	.82	47.33
2	-.48	-36.09	1.56	81.30
3	.44	46.81	.68	99.85
4	-1.23	-69.49	3.28	73.43
5	.96	109.09	.48	89.10
6	.83	345.83	.22	82.68
7	.56	43.41	.70	253.15
8	.57	24.05	.81	172.28
9	.00	0	0	0
10	.59	0	.00	31.98
		Tinnitus		
1	-.069	-66.35	2.97	28.73
2	.77	102.67	.49	81.33
3	.19	176.19	.36	24.44
4	.66	4366.67	.02	28.74
5	.01	2.17	.98	38.83
6	1.65	366.67	.21	135.98
7	-3.11	-100	0	14.97
8	1.20	200	.33	182.85
9	2.30	0	.00	188.05
10	.00	0	0	28.65

The results of the regression analysis yielded no significant difference in the melatonin ratio ($F(1, 12)=1.05$, $p=.325$), melatonin increment ($F(1, 15)=.599$, $p=.451$), and melatonin percentage ($F(1,14)=1.19$, $p=.297$) reactivity measures after controlling for the effects of sleep, stress, tinnitus severity, and the 0min melatonin baseline measure. The same regression model used the melatonin AUC as the dependent variable, with the tinnitus vs. control groups as the fixed variables. Similarly, the analysis

revealed that the melatonin AUCs of between-subjects' tests yielded no significant difference (F (1,15)=1.16, p=. 298) after controlling for sleep, stress, tinnitus severity, and melatonin baseline measure.

Assessment of Differences at Each Measurement Point

In order to examine changes in the mean scores under the four different time points, repeated measures and a one-way ANOVA using the baseline, 5 min, 30 min, and 60 min posttest time points as the within-subjects variable and the subjects group, control vs. tinnitus, as the between-subjects factor. Figure 19 illustrates the mean melatonin values across the different time intervals.

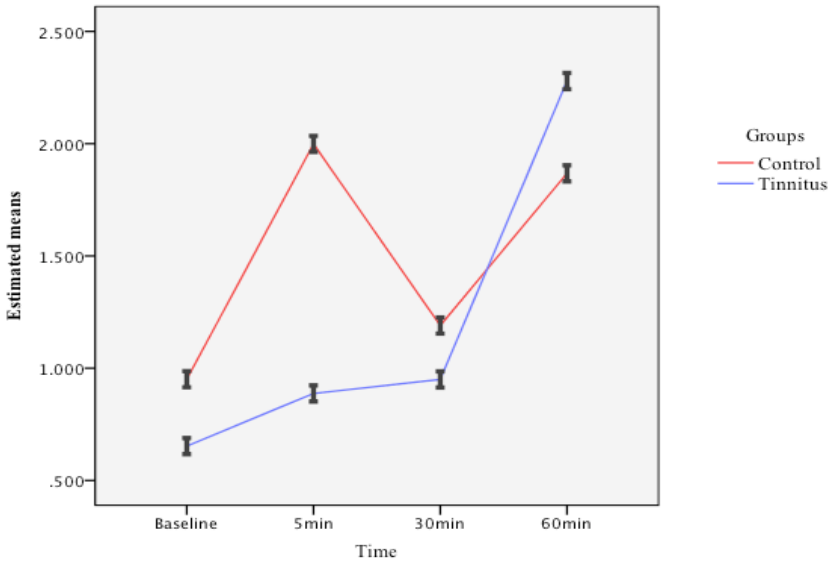

Figure 19. Melatonin Mean Values across Different Time Intervals.

Table 28 shows mean, standard deviation and standard errors of the melatonin values at the different time points. Similar to the salivary alpha-amylase results, the tinnitus started with lower melatonin values at baseline (M=. 653, SD=. 933) that

increased in mean of 0.23 rights after introducing the stressor at 5 min posttest (M=. 887, SD=. 952). Nevertheless, the tinnitus group showed overall lower values cross the different time intervals compared to the controls, with the exception of 60 min posttest when the tinnitus group was higher in mean values (M=2.279, SD=2.58) compared to the controls (M=1.86, SD=2.17).

Table 28

Melatonin at Different Time Intervals

Time		*M*	*SD*	*SE*
Baseline	Control	.951	.769	.243
	Tinnitus	.653	.933	.295
5 min	Control	1.999	1.894	.598
	Tinnitus	.887	.952	.301
30 min	Control	1.190	.843	.266
	Tinnitus	.950	.888	.280
60 min	Control	1.868	2.174	.687
	Tinnitus	2.279	2.585	.817

Both the control and tinnitus groups seemed to follow a similar pattern of lower reaction baseline values that increased over time. The greatest difference in the mean between the two groups was observed at 5 minutes posttest when the tinnitus group exhibited lower mean values (M=. 887, SD=. 952) compared to the controls (M=1.99, SD=1.89). Furthermore, the results of the one-way ANOVA F-test yielded no significant difference between the baseline (F (1,18)=. 607, p=. 446), 5 min (F (1,18)= 2.74, p=.

115), 30 min (F (1,18)=. 384, p=. 543) and 60 min posttest (F (1,18)=. 148, p=. 705) time intervals.

In the repeated-measures ANOVA conducted, the main effect of time was found to achieve statistical significance (F (3, 54) = 3.705, p = .036), indicating significant within-subjects effects. The main effect of participant group failed to achieve statistical significance (F (1, 18) = .424, p = .523). Finally, the interaction between time and group membership was also not found to achieve statistical significance (F (3, 54) = 1.187, p = .316).

A boxplot and melatonin standardized residuals were computed to identify the presence of any outliers (figures 20 & 21). Figure 20 indicates one subject in the control group, number 7, may have influenced the means of the two groups. The previous analyses were repeated, omitting this one observation, and the conclusions remained unchanged.

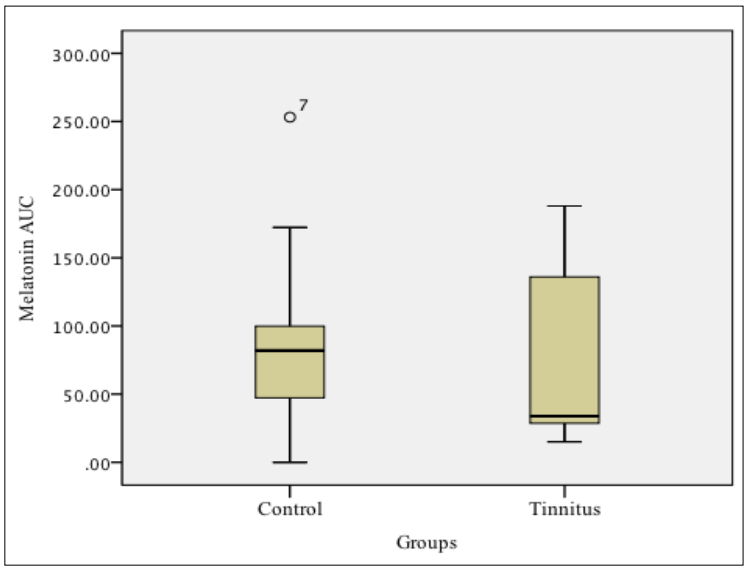

Figure 20. Melatonin Boxplot.

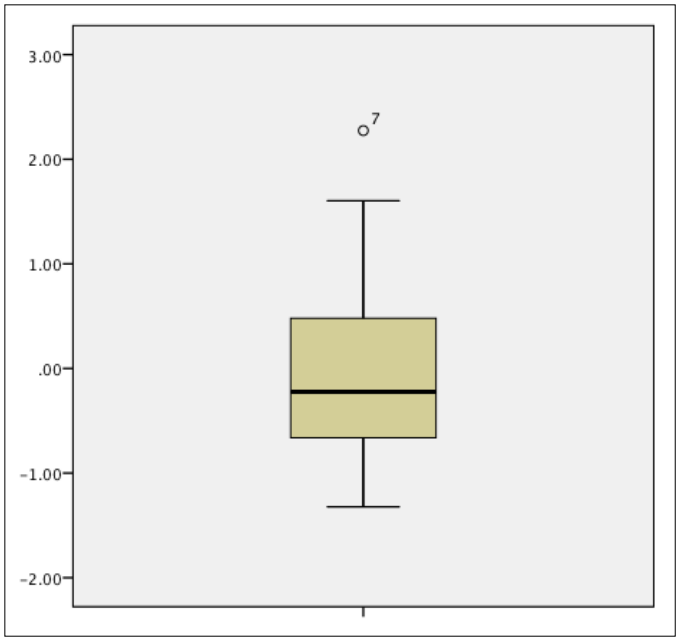

Figure 21. Melatonin Standardized Residuals.

The assumption of normality was tested via examination of standardized residuals. The pattern revealed through the box plots of melatonin data does not indicate a serious departure from normality.

Kruskal-Wallis and One-Way ANOVA Test Results

Although serious departures from normality were not evident, nonparametric Kruskal-Wallis tests were computed. Table 29 and 30 show the results of the Kruskal-Wallis tests for melatonin.

The nonparametric test revealed no statistically significant difference in the melatonin levels across the different time intervals between the two groups, control vs. tinnitus. As observed in table 30, the p(sig) for the four different time intervals was insignificant and ranged from (x^2 =1.295, p=. 255, p<. 0125) at baseline, (x^2 =3.023, p=. 082, p<. 0125) at 5 min, (x^2 =. 694, p=. 405, p<. 0125) at 30 min, and (x^2 =. 051, p=. 821,

p<. 0125) at 60 min posttest. Nevertheless, the results of the Kruskal-Wallis test were found to be consistent with the ANOVA test results (table 31). The analysis of variance showed that there was no significant effect of melatonin values at the different time intervals.

Table 29

Melatonin Mean Ranks

Time	Group	Mean rank
Baseline	Control	12.00
	Tinnitus	9.00
5 min	Control	12.80
	Tinnitus	8.20
30 min	Control	11.60
	Tinnitus	9.40
60 min	Control	10.20
	Tinnitus	10.80

Table 30

Melatonin Kruskal-Wallis Test Results

Melatonin	Baseline	5 min	30 min	60 min
Chi-Square	1.295	3.023	.694	.051
df	1	1	1	1
Asymp. Sig.	.255	.082	.405	.821

Note. Bonferroni correction applied: p (sig)=0.0125.

Table 31

Melatonin ANOVA Test Results

Melatonin	Baseline	5 min	30 min	60 min
F-value (1,18)	.607	2.74	.384	.148
p (sig)	.446	.115	.543	.705

Melatonin Differences from Baseline

Table 32 shows the results of the subtracted melatonin mean values at 5 min, 30 min, and 60 min posttest from the baseline in both the control and tinnitus groups. The results show that both groups seem to follow a similar pattern of reaction, with the lower values at 5 min in the tinnitus group (M=. 234, SD=1.376) compared to the controls (M=1.04, SD=1.826) increasing over time with the highest increase in the tinnitus group (M=1.62, SD=3.024) at 60 min posttest, compared to (M=. 0917, SD=1.821) in the controls.

Table 32

Melatonin Differences from Baseline

Time	Groups	*M*	*SD*	*SE*
5 min	Control	1.048	1.826	0.577
	Tinnitus	0.234	1.376	0.433
30 min	Control	0.239	0.665	0.210
	Tinnitus	0.297	1.48	0.468
60 min	Control	0.917	1.821	0.576
	Tinnitus	1.626	3.024	0.956

A one-way ANOVA F-test revealed no significant difference between the melatonin measures at 5 min (F (1,18)=1.265, p=0.275, p<. 016), 30 min (F (1,18)=. 013, p=. 911, p<. 016), and 60 min (F (1,18)=. 403, p=. 533, p<. 016) posttest. Furthermore, the results of the parametric one-way ANOVA were consistent with the results of the nonparametric Kruskal-Wallis test (tables 33 and 34) that also showed no significant difference between the 5 min (x^2 =. 966, p=. 326, p<. 016), 30 min (x^2 =. 242, p=. 623, p<. 016), and 60 min posttest (x^2 =. 572, p=. 450, p<. 016) time measures.

Table 33

Melatonin Differences ANOVA Test Results

Melatonin	5 min	30 min	60 min
F-value (1,18)	1.265	0.013	0.403
p (sig)	0.275	0.911	0.533

Table 34

Melatonin Differences Kruskal-Wallis Test Results

Melatonin	5 min	30 min	60 min
Chi-Square	0.966	.242	0.572
df	1	1	1
Asymp. Sig.	0.326	0.623	0.450

Note. Bonferroni correction applied: p (sig)=0.016.

Neopterin Data

Assessment of Overall Group Differences

Table 35 shows increment, ratio, and AUC values for Neopterin reactivity to a 5-minute stressor in 20 subjects. Saliva samples for neopterin assay were collected at 0min

baseline, 5 min right after the stressor, and at 30 and 60 minutes after the end of the stressor.

Table 35

Neopterin Reactivity

#	Increment	%	Ratio	AUC
		Control		
1	-.09	-13.43	1.16	36.53
2	-.05	-5.88	1.06	48.28
3	.02	1.53	.98	74.80
4	-.03	-4.17	1.04	41.48
5	-.02	-3.23	1.03	36.50
6	.04	1.64	.98	149
7	.04	1.72	.98	139.55
8	.31	13.60	.88	147.88
9	-.28	-8.19	1.09	187.90
10	.10	3.97	.96	1540.70
		Tinnitus		
1	-.05	-7.94	1.09	34.48
2	-.53	-35.33	1.55	61.47
3	.01	.33	1	187.63
4	-.17	-5.43	1.06	172.48
5	.71	27.52	.78	180.02
6	.34	12.27	.89	186.05
7	-.61	-24.30	1.32	125.58
8	-.56	-22.49	1.29	124.10
9	-.14	-5.13	1.05	158.90
10	.01	.45	1	131.08

To examine changes in the mean scores under the four different reactivity measures of increment, ratio, percentage, and AUC, a regression analysis model was fitted using the four measures of reactivity as the within-subjects dependent variable and the subjects group, control vs. tinnitus, as the between-subjects factor, after controlling

for the effects of sleep, stress, and baseline measure of the neopterin biomarker. Figure 22 illustrates the mean neopterin levels across the different reactivity measures.

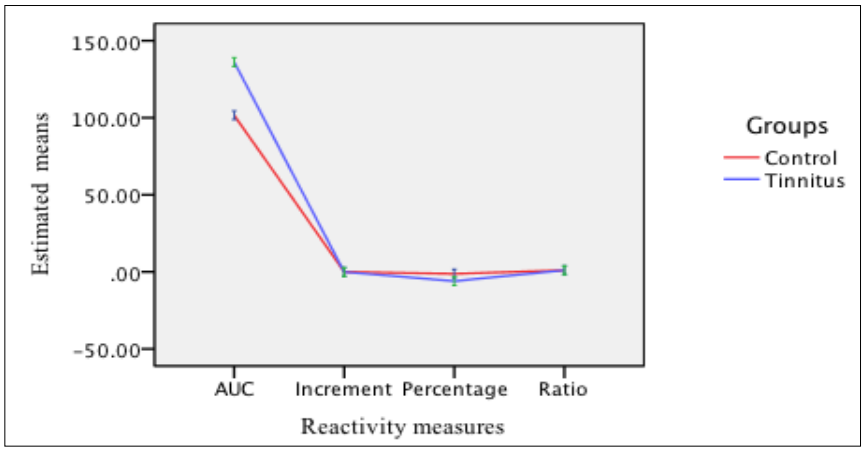

Figure 22. Neopterin Reactivity Measures.

The results of the regression analysis yielded no significant difference in the neopterin ratio (F (1, 15)=2.69, p=. 122), neopterin increment (F (1, 15)=. 907, p=. 356), and neopterin percentage (F (1,15)=1.99, p=. 178) reactivity measures after controlling for the effects of sleep, stress, tinnitus severity, and 0min neopterin baseline measure. The same regression model used the neopterin AUC as the dependent variable, with the tinnitus vs. control groups as the fixed variables. Similarly, the analysis revealed that the neopterin AUCs of between-subjects' tests yielded no significant difference (F (1,15)=. 846, p=. 372) after controlling for sleep, stress, tinnitus severity, and neopterin baseline measure. Although the regression analysis showed no statistical significance, the tinnitus group's neopterin AUC mean value (M=136.17, SD=52.80) was increasingly bigger than that of the controls' (M=101.66, SD= 59.39) mean value.

Assessment of Differences at Each Measurement Time

To measure changes in the mean scores at the four different time points, a repeated-measures ANOVA model was fitted using the four time points, baseline, 5 min, 30 min, and 60 min posttest, as the within-subjects variable and the control vs. tinnitus groups as the between-subjects factor.

Table 36 shows the mean, standard deviation, and standard errors of the neopterin values at the different time points. As shown in figure 23, the neopterin pattern of reaction is characterized by higher values at baseline (M=2.35, SD=. 757) compared to (M=1.71, SD=. 998) in the control group. The neopterin values exhibited a slight decrease after the stressor was introduced at 5 min (M=2.29, SD=. 844), 30 min (M=2.25, SD=. 924), and 60 min (M=2.24, SD=902) in the tinnitus group. Although both groups displayed the same form of decrease after the stressor was presented, the tinnitus group values were higher at each of the time points when compared to the controls.

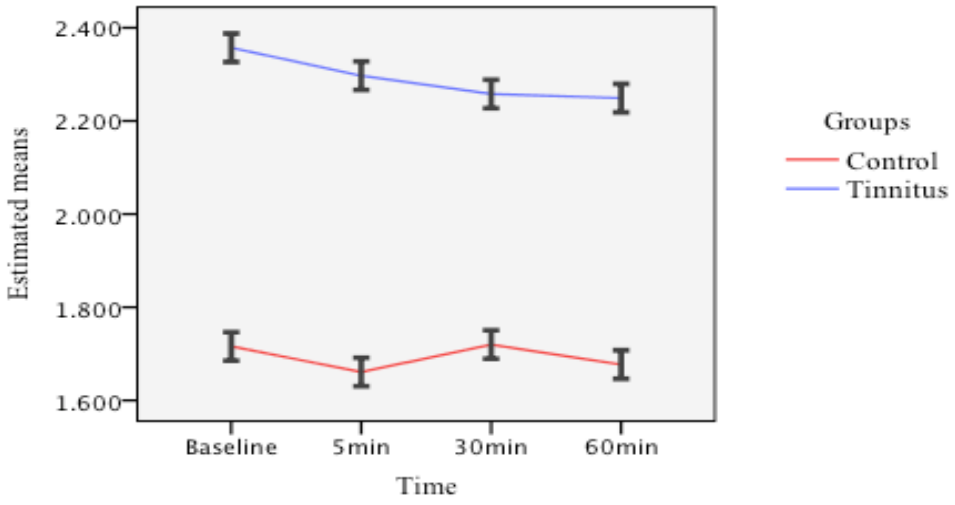

Figure 23. Neopterin Mean Values across Different Time Intervals.

Table 36

Neopterin at Different Time Intervals

Time		*M*	*SD*	*SE*
Baseline	Control	1.716	.998	.315
	Tinnitus	2.357	.757	.239
5 min	Control	1.661	.967	.305
	Tinnitus	2.297	.844	.267
30 min	Control	1.720	1.01	.319
	Tinnitus	2.258	.924	.292
60 min	Control	1.677	.993	.314
	Tinnitus	2.249	.902	.285

Additionally, a one-way ANOVA F-test yielded no statistical significance at baseline ($F(1,18)=2.61$, $p=1.23$), 5 min ($F(1,18)=2.45$, $p=.135$), 30 min ($F(1,18)=1.54$, $p=.230$), and 60 min ($F(1,18)=1.81$, $p=.194$) posttest measures.

In the repeated-measures ANOVA conducted, the main effect of time was not found to achieve statistical significance ($F(3, 54) = .678$, $p = .505$), indicating no significant within-subjects effects. The main effect of participant group also failed to achieve statistical significance ($F(1, 18) = 2.119$, $p = .163$). Finally, the interaction between time and group membership was also not found to achieve statistical significance ($F(3, 54) = .428$, $p = .643$). Figures 24 and 25 indicate subject number 11 in the tinnitus group may have influenced the mean. The previous analyses were repeated, omitting this one observation, and the conclusions remained unchanged. The assumption of normality was tested via examination of the standardized residuals. The pattern

revealed by the box plots and of neopterin data does not indicate a serious departure from normality.

Kruskal-Wallis and One-Way ANOVA Test Result

Although serious departures from normality were not evident, nonparametric Kurskal-Wallis tests were computed. Tables 37 and 38 show the results of the Kruskal-Wallis tests for neopterin.

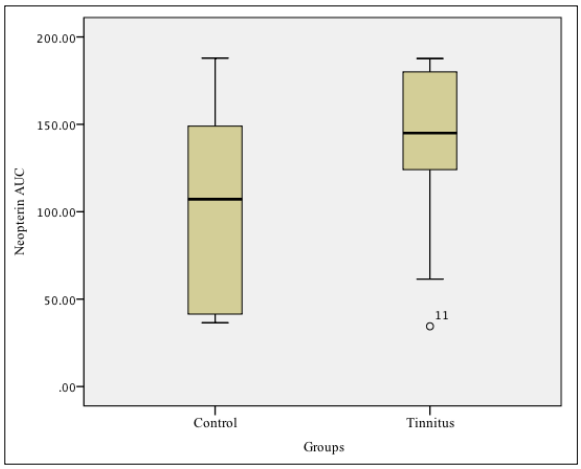

Figure 24. Neopterin Boxplot.

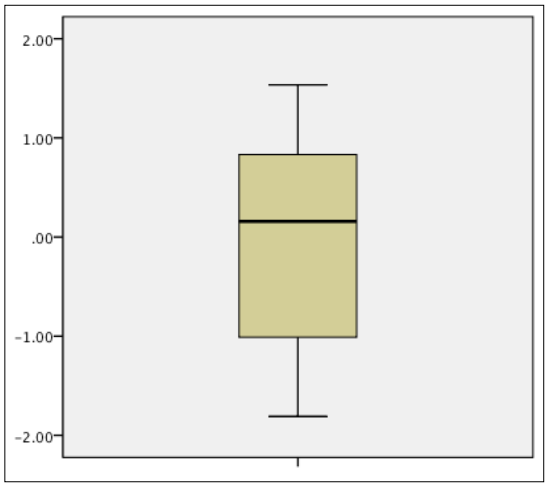

Figure 25. Neopterin Standardized Residuals Boxplot.

Table 37

Neopterin Mean Ranks

Time		Mean rank
Baseline	Control	8.30
	Tinnitus	12.70
5 min	Control	8.75
	Tinnitus	12.25
30 min	Control	9.10
	Tinnitus	11.90
60 min	Control	9.00
	Tinnitus	12.00

Table 38

Neopterin Kruskal-Wallis Test Results

Neopterin	Baseline	5 min	30 min	60 min
Chi-Square	2.766	1.754	1.122	1.286
df	1	1	1	1
Asymp. Sig.	.096	.185	.290	.257

Note. Bonferroni correction applied: p (sig)=0.0125.

The nonparametric test revealed no statistically significant difference in the neopterin values across the different time intervals between the two groups, control vs. tinnitus. As observed in table 38, the p(sig) for the different four time intervals was insignificant and ranged from (x^2 =2.76, p=. 096, p<. 0125) at baseline, (x^2 =1.754, p=. 185, p<. 0125) at 5 min, (x^2 =1.12, p=. 290, p<. 0125) at 30 min, and (x^2 =1.28, p=. 257, p<. 0125) at 60 min posttest. Nevertheless, the results of the Kruskal-Wallis tests were found to be consistent with the ANOVA test results shown in table 39. Similarly, the

analysis of the ANOVA variance showed that there was no significant effect of neopterin values at baseline (F (1,18)=2.61,p=. 123), 5 min (F (1,18)=2.45, p=. 135), 30 min (F (1,18)=1.54, p=. 230, and 60 min posttest (F (1,18)=1.81, p=. 194).

Table 39

Neopterin ANOVA Test Results

Neopterin	Baseline	5 min	30 min	60 min
F-value (1,18)	2.61	2.45	1.54	1.81
p (sig)	.123	.135	.230	.194

Neopterin Differences from Baseline

Table 40 shows the subtracted neopterin mean values at the 5 min, 30 min, and 60 min posttest time values from the baseline in both groups, control vs. tinnitus. The results show that overall; the tinnitus group exhibited a smaller decrease in mean values from baseline when compared to the controls.

Table 40

Neopterin Differences from Baseline

Time	Groups	*M*	*SD*	*SE*
5 min	Control	-0.055	0.089	0.028
	Tinnitus	-0.060	0.233	0.0737
30 min	Control	0.004	0.149	0.047
	Tinnitus	-0.099	0.413	0.130
60 min	Control	-0.039	0.228	0.072
	Tinnitus	-0.108	0.336	0.106

Table 41

Neopterin Difference ANOVA Test Results

Neopterin	5 min	30 min	60 min
F-value (1,18)	.004	.594	.289
p (sig)	.950	.468	.598

Table 42

Neopterin Differences Kruskal-Wallis Test Results

Neopterin	5 min	30 min	60 min
Chi-Square	.000	1.378	.894
df	1	1	1
Asymp. Sig.	1.000	.241	.344

Note. Bonferroni correction applied: p (sig)=0.016.

A one-way ANOVA F-test revealed no significant difference between the melatonin measures at 5 min (F (1,18)= .004, p=. 950, p< .016), 30 min (F (1,18)=. 594, p=. 468, p<. 016), and 60 min (F (1,18)=. 289, p=. 598, p<. 016) posttest (table 41). Furthermore, the results of the parametric one-way ANOVA were consistent with the results of the nonparametric Kruskal-Wallis test (table 42), which also revealed no significant difference at the 5 min (x^2 =. 00, p=1, p<. 016), 30 min (x^2 = 1.378, p=. 241, p<. 016), and 60 min posttest (x^2 =. 894, p=. 344, p<. 016) time intervals.

CHAPTER V

DISCUSSION

The aim of this study was to:

1. Document specific subcortical hormonal areas of influence on the experience of chronic tinnitus by measuring the baseline of four stress-related hormone levels:
    a. Circadian cycle with Melatonin,
    b. Stress with Cortisol,
    c. Stress with Salivary Alpha-Amylase, and
    d. Inflammatory immune system and Neopterin as a measure of immune system reactions;
2. Investigate whether hypothalamic regulation of responses to stress, as reflected in the level of these four stress-related hormones, is greater in male participants with chronic tinnitus; and
3. Utilize the results to support a preliminary framework for a hypothetical T-NPIE model of chronic tinnitus that will lead to future investigation of underlying tinnitus factors originating from any disruption in the autonomic nervous system (ANS), the hypothalamic pituitary adrenal (HPA) axis regulation of stress, and the inflammatory reactions of the immune system.

A group of 20 adult male subjects, ten with tinnitus and ten without tinnitus, were included in this study. Prior to determining enrollment eligibility, all the subjects were phone-screened and asked specific questions about their general health, diet, medication, physical well-being, and sleep patterns. Surveys were sent out, and the subjects were instructed to bring all the survey forms to their scheduled lab visits for further hearing testing and saliva samples collections.

The subjects of this study were adult males ranging in age from 18 to 31 years (M=23.6 years, SD=4.47). Hearing threshold tests revealed that hearing was not a significant factor; all the subjects had normal hearing of 20 dB HL or less. Similarly, pure tone averages of the two groups were not significant (RE: F (1,18)=0. 508, p=0. 485; LE: F (1,18)=2.77, P=0. 113).

Tinnitus was gradual in onset (50%, n=5) in the half of the tinnitus subjects and characterized with pitch in the mid-3000Hz, with lower loudness levels that ranged from 13 to 58 dB HL. The majority (50%, n=5) reported their tinnitus lasted in duration from one to five years.

This study did not control for race, which was not considered to be an exclusionary factor. The findings of this study reported that race was normally distributed in the tinnitus group, which was mainly white, but not in the control group, with the majority being African American. Tucker, et al. (2005), in a sample of 120 normal hearing individuals, reported tinnitus was most commonly perceived among Caucasians (78%) than African Americans (38%) and the difference was significant (x^2 =22.19, p= 0.001). The relationship between race and tinnitus is a rear-investigated topic; and in

spite of reports of clinical observations of the influence of race on the experience of tinnitus, few studies have actually documented the influence of racial differences on tinnitus severity.

As for diet, the subjects' dietary styles were characterized overall as high on carbohydrates and low on antioxidants. Out of the ten-tinnitus subjects, 70% stated their diet was low on antioxidants and 90% stated their diet was high on carbohydrates. These dietary styles could possibly explain the higher neopterin mean values observed in the tinnitus group.

Oxidants are free radicals that are formed from oxygen reactive oxygen species (ROC). They are implicated in pathologies of the inner ear and the peripheral and central pathways (Clerici and Yang, 1996; Neri et al., 2002). Antioxidants work against the oxidants in the body and attempt to repair the cell damage caused by the harmful oxidants. Antioxidants, as well as minerals and other remedies such as herbal have been used before as possible tinnitus treatment options (Enrico et al., 2007). A diet that is high in antioxidants in general can influence some hormonal functions. This area of investigation is growing rapidly, with evidence pointing toward the emergence of possible pharmacological and herbal treatment options for subjects with tinnitus.

Behavioral Measures

Tinnitus Severity and Tinnitus History (TSI, THQ) Questionnaires

Tinnitus severity can be quantified in different ways. This study used the Tinnitus Severity Index (TSI) and Tinnitus History Questionnaire (THQ) to assess how negatively tinnitus affects an individual's life and how bothersome it was perceived to be (Meikle,

Griest, and Stewart, 1995). Regardless of the type of assessment tool used to quantify the severity of tinnitus, most of these tools ask questions that aim to subjectively describe how bothersome tinnitus is and the effect it has on an individual's quality of life.

It is common to have a discrepancy in the way tinnitus is subjectively reported and objectively measured (Folmer & Stevenson, 2002), which could result from subjects not understanding the questions. Since the majority of this study's subjects had tinnitus for one to five years (50%) or for more than five years (40%), it is possible they eventually developed adaptation strategies that helped them overcome the bothersome effects of tinnitus.

Very often, TSI test results correlated with subjective measures of tinnitus loudness. For example, Question 19 on the THQ states, "On the scale, circle the number that best describe the loudness of your usual tinnitus" (Folmer & Stevenson, 2002). Results of the tinnitus severity index in this study reveal that there is a significant positive correlation between the subjective rating of tinnitus loudness on the tinnitus history questionnaire and tinnitus severity index ($r= .0889$, $n=10$, $p= .001$, $p<. 05$).

Ultimately, the goal of these questionnaires is to describe how bothersome tinnitus is for an individual and to help educate them about their tinnitus to the point where tinnitus is no longer bothersome. This is mostly done in a longitudinal manner. Individuals are assessed every 3 or 6 months, and results of their initial TSI are used to compare and contrast changes in the way tinnitus severity is reported and to provide a better tinnitus management plan (Lindberg et al.1989; Scott et al., 1985; Ireland et al.,

1985). This study only assessed tinnitus severity at one point in time. Comparisons over different point of time were not made.

The Perceived Stress Scale (PSS)

This study assessed the subjects' generalized perception of stress by using the PSS 14-items questionnaire. Subjects were also asked to subjectively rate how stressed they were on a scale from zero to 10. Previous reports have shown that stress exacerbates the severity and intensity of tinnitus perception (Henry & Wilson, 2001; Nodar, 1996) and can radically contribute to worsened tinnitus symptoms (Schmitt et al., 2000).

Correspondingly, stress was assumed to modulate tinnitus and to be heightened in individuals with tinnitus (Hebert & Lupien, 2007). The results of this study are in line with other reports (Henry & Wilson, 2001; Nodar, 1996; Hebert & Lupien, 2007; Moller, 2006; Schnitt et al., 2000) indicating a higher subjective rating of stress levels in individuals with tinnitus. According to the PSS scores of this study, subjects with tinnitus had overall higher reports of stressful events in their lives, with scores of 20 and more. Scores around 13 are considered average, while scores around 20 and higher suggest higher stress levels with 40 indicating the highest level of stress. PSS Norms were based on an L. Harris Poll that gathered information from 2387 respondents in the United States (Cohen et al., 1988). The results of this study showed that the PSS was statistically significant ($F(1,18)=13.50$, $p= .002$, $p< .05$), with six (60%) of the ten tinnitus subjects categorized to have had higher stress levels when compared to the healthy controls. In addition, the raw mean values of the tinnitus group were higher ($M= 20.30$, $SD=6.86$) than the norms of the same age range ($M=14.2$, $SD=6.2$) when

compared to the data obtained from the 2387 respondents. Overall, higher stress scores were associated with greater vulnerability to stressful life events (Cohen & Williamson, 1988; Cohen et al., 1991; Glaser et al., 1999).

On the other hand, in term of constructed validity, PSS scores tend to correlate with subjective measures of stress (Cohen et al., 1988). Correspondingly, this study found that there was a positive linear correlation between the PSS overall scores and subjective rating of stress ($r= 0.885$, $n=20$, $p<0.001$). This finding is consistent with other reports indicating that PSS correlates with a range of self-reported behavioral data (Sheldon et al., 1983; 1988).

Similar to the TSI, high scores of PSS are used as an inductive factor for future distress and to predict sensitivity to stress, especially if administrated repeatedly (Cohen et al., 1983). Results of the PSS are influenced by changes in daily activities or major life events and are expected to increase or decrease rapidly after 4 to 8 weeks (Cohen et al., 1988). The PSS scores reported in this study were limited to one point in time. Comparisons of changes over longer periods were not made. Moreover, it is worthwhile to note that this study's sample size was relatively small and gender was limited to male subjects only, which may have influenced the observed change in mean values across the two groups.

Pittsburgh Sleep Quality Index (PSQI)

The PSQI has been shown to be a valid measure of sleep quality (Buysse et al., 1989; Backhaus et al., 2002). Sleep disturbance is a common complaint among people with tinnitus. Overall studies have reported that sleep problems are more common in

subjects with tinnitus than subjects without tinnitus (Folmer & Griest, 2000; Riter, 2003). Similarly, the findings of this study showed that there was a significant difference in sleep disturbances between the two groups ((F (1,18)=6.818, p=0. 018). The majority of the tinnitus subjects (60%) scored higher on the PSQI (M=4.9, SD=2.1) and consequently were assigned as having poor sleep quality compared to the controls (M=2.7, SD=1), whom almost all (90%), except for one subject, were assigned to the good sleep quality group. Moreover, previous reports have shown that sleep disturbances in subjects with tinnitus, although partially independent from complaints such as distress (Hallam, 1996; Hiller & Goebel, 1992), correlated highly with other psychological factors such as depression (Alster, Schemsh, & Ornan, 1993) and the tinnitus severity (Folmer & Griest, 2000).

The findings of this study also report a positive linear correlation between sleep disturbances and stress ($r= 0.892$, $n=20$, $p<0.001$) in all subjects and between sleep disturbances and tinnitus severity ($r=. 741$, $n=10$, $p<0.014$) in the tinnitus subjects. Similar to TSI and PSS, the PSQI proved to be a valid measure to predict sleep disturbances, especially if administrated repeatedly (Uchechukwu et al., 2006). The PSQI was administrated once at this study, so comparisons of change over time based on changes in the PSQI scores in tinnitus subjects were not possible.

The International Physical Activity Questionnaire (IPAQ)

The IPAQ is a reliable measure that uses categorical and continuous data to categorize subjects into one of three main categories: inactive, minimally active, and health-enhanced physical activity (Booth et al., 2003). Very few studies have investigated

the role a subject's particular physical activity has on the intensity and severity of their tinnitus (Jake Richard, 2012-2013, ATA funded grant). On the other hand, relaxation has shown to effectively decrease tinnitus annoyance (Jakes et al., 1986). Overall, this study found that all of the subjects, both with and without tinnitus, had similar physical activity levels: a minimal physical activity life style, with the majority reporting 5 or more days of walking at least 30 minutes per day as their primary physical activity. Physical activity and exercise have been found to positively influence neural plasticity and cognition (Hotting & Roder, 2013), oxidative stress (Radak et al., 2008), depression and anxiety (Byrne & Byrne, 1993), and sleep (Driver & Tayloer, 2000). With that in mind, all of the above investigated areas, such as sleep, depression, stress, cognition, neural plasticity, and anxiety, are complaints reported by tinnitus subjects, We can only assume that exercise and physical activity will have the same positive influence on tinnitus intensity and severity. Future research should aim to further examine the role of exercise on tinnitus intensity.

Cortisol

The present study hypothesized that subjects with tinnitus would experience higher cortisol responses and slower recovery time to a stressor. Accordingly, the findings of the cortisol raw and mean data suggest evidence of a potential difference between cortisol baseline and 5 minutes posttest between the two groups, control vs. tinnitus. Although the majority of our subjects with tinnitus rated their stress as higher at the four different collection times, there was no statistical significance in ratings of subjective stress between the two groups. This finding is consistent with Hebert and

Lupien's (2007) report of no difference in stress rating, but our finding differs from the findings of Heuneck et al. (2008), where subjects with tinnitus rated their stress to be higher than the control subjects.

Cortisol values in the control group exhibited a pattern of reaction that was similar to that reported in prior investigations. Cortisol values increased right after introducing the stressor and decreased over time to a point closer to the initial baseline measure. Prior investigations reported that cortisol exhibited a similar reaction in healthy individuals (Hebert and Lupien, 2009; Hebert and Lupien, 2007). In the subjects with tinnitus, cortisol reactions seemed to be slower and more blunt in effect (Hebert and Lupien, 2007). In Hebert and Lupien's (2009) findings, however, cortisol levels increased steadily and peaked at 30 minutes after the stressor or noise was introduced and then dropped off when the stressor was removed. This results of our study partially corresponded to their findings. In our study, the cortisol peak time was faster at 5 minutes posttest; but both groups had a steady decrease in cortisol values after the stressor was introduced.

In the Herbert and Luipen's (2006) investigation, the reported blunted cortisol responses in the subjects with tinnitus were not related to the presence or clinical diagnosis of any major psychological disorder such as depression or anxiety. Such conditions would considerably activate hormonal secretions of the HPA axis; however, the absence of such diagnosis in our subjects with tinnitus might suggest that their slower and dampened cortisol responses could result from a predisposed tinnitus-related mechanism.

Nevertheless, slower reactivity to cortisol has been reported in "some" individuals with other chronic stress health conditions such as chronic fatigue (Roberts et al., 2004), depression (Gold & Choruses, 2002), chronic pelvic pain (Heim et al., 1998), fibromyalgia (Gur et al., 2004), and schizophrenia (Dinan, 2004). Equally, tinnitus is a condition that in many circumstances can be characterized as an enhanced chronic stress trigger (Henry & Wilson, 2001; Nodar, 1996). As observed in this study, subjects with tinnitus exhibited a similar, if not the same, mechanism of chronic stress conditions. Different investigations related such responses to inhibition of the HPA axis through enhanced negative feedback sensitivity (Moller et al., 1992), to mechanisms that involved regulation of the non-classical auditory pathways (Muhlau et al., 2006), or to brain structures such as the amygdala, that are also involved in conditions such as chronic pain (Muhlau et al., 2006; Moller, 1997).

Surprisingly, although recovery was slower for the subjects with tinnitus, both the control and tinnitus groups exhibited a very similar general "lessening phase" after the stressor was introduced. These findings might reflect the tinnitus group's ability to adapt to the nature of the stress from having experienced chronic tinnitus over time. This suggests that when faced with stress resulting from a chronic condition such as tinnitus, individuals adapt their responses to the chronic nature of the stressor, which results in a lower production of stress-related hormones. Lower cortisol levels can also suggest that in cases of prolonged chronic stress, the cortisol hormone is overused and less available in the tinnitus group.

The findings of this study support the hypothesis of hyperactivity of the autonomic nervous system in subjects with tinnitus. Future studies should further investigate the role of the HPA axis on the regulation of tinnitus severity and intensity and the different mechanisms of cortisol reactivity in subjects with tinnitus to enable us to better develop a model that proposes different yet promising tinnitus treatments.

Salivary Alpha-Amylase (sAA)

While cortisol is involved in the regulation of stress responses modulated by the HPA axis, the salivary alpha-amylase response to stress is related to the activity of the sympathetic adernomedullary (SAM) system. Furthermore, it was long believed that not the SAM system but the HPA axis is responsible for distinct responses to stress. Consequently, the role of sAA in the regulation of stress was overlooked for a while.

Studies have found that salivary alpha-amylase increases in response to a state of stress when the autonomic nervous system activity increases (Nater and Rohleder, 2009; Gilman et al., 1997a; Chatterton et al., 1997; Chatterton et al., 1996; Bosch et al., 1996). Unlike cortisol, a well-known and documented marker of stress, the idea of documenting the use of sAA as a marker of psychological stress is recent. Increases in sAA values are involved in both sympathetic (fight-or-flight) responses and parasympathetic (e.g., saliva secretions) stimulations of the autonomic nervous system (Ehlert et al., 2006; Nater and Rohleder, 2009).

To date, there are no studies that investigate the correlation between salivary alpha-amylase levels and chronic tinnitus; however, a significant increase in sAA was found in relation to traffic noise exposure. Wagner et al. (2010) examined twenty subjects

who were exposed to binaural traffic noise with levels of 75dB for 20 minutes. The researchers collected saliva samples for cortisol and salivary alpha-amylase right after and before the noise exposure. Their findings showed increased levels of sAA and cortisol concentration after the noise exposure ($p=0.045$, $p=0.01$). The findings of this study propose the probability of using sAA as a stress marker in relation to induced noise.

The present study hypothesizes subjects with tinnitus would experience higher sAA responses and slower recovery time in response to a stressor. The findings of this study suggest evidence of a potential difference in sAA levels at baseline, 30, and 60 minutes posttest between the two groups, control vs. tinnitus, with lower sAA levels across all time points in the subjects with tinnitus.

Prior investigations have found that salivary alpha-amylase is sensitive to psychological (Nater et al., 2005, 2006) and physiological stress (Chatterton et al., 1996). The preliminary findings of the current investigation show that there is a marked decrease at the 5 minutes post stressor in sAA in the healthy controls. This finding is inconsistent with other investigators who reported an increase in sAA values after exposure to short periods of acute psychological stress in healthy controls (Kirschbaum et al., 1993; Nater et al., 2005, 2006). More specifically, a marked increase in sAA has previously been reported in healthy controls after exposure to a designated stress-inducing, counting backwards task (Noto et al., 2005). Surprisingly, this study found a marked *decrease* in sAA in healthy controls after exposure to a stress-inducing, counting backwards task. These conflicting results could possibly be explained by differences in the experimental

and group designs. For example, while some reported increases in sAA right after a mental arithmetic task, others reported no change in sAA levels (Borgeat et al., 1984).

The subjects with tinnitus displayed a different reaction pattern at baseline, 5, and 30 minutes post stressor than that of the controls. The tinnitus group sAA values were overall lower than the controls' sAA values. Over time, the sAA values slightly increased, which suggests a potentially smaller response to stress. Interestingly, this finding is consistent with prior investigations (Kirschbaum et al., 1993; Nater et al., 2005, 2006) that show an increase in sAA right after introducing the stress. This finding is consistent with the findings of Nater et al. (2005), who reported a peak of sAA activity immediately after an acute stressor, but inconsistent with the findings of Morse et al. (1981c), who reported fluctuations in the mean sAA.

There is a paucity of research in regard to sAA changes and tinnitus, and the results of sAA studies in general have been found to be inconsistent (Nater et al., 2005). The finding of this study showed that sAA responses in the subjects with tinnitus seemed to follow the opposite reaction of the controls. While sAA increased after the stressor was introduced in the subjects with tinnitus, the controls exhibited a decreased reaction and vise versa. Moreover, although cortisol and sAA are regarded as stress markers, the cortisol baseline measure or mean value is higher than the sAA baseline measures in the subjects with tinnitus when compared to the controls. This study was designed to test for a correlation between the two physiological measures. Surprisingly, except for 30 min posttest, no correlation was found between the two measures at the other three time points. At 30 min posttest, a positive linear correlation was computed (r= .744, n=20, p=.

033, p< .05) between the cortisol and sAA mean values. Furthermore, the sAA AUC was found to negatively correlate with this study's measure of stress (r=-0.465, n=20, p=. 039, p< .05) but not sleep or tinnitus severity.

Based on the findings of prior investigations and the suggestive potential evidence of this study, it is hypothesized that sAA could be used as a biological marker for acute and chronic stress brought on by tinnitus. Any discrepancy in the findings may be explained by the fact that stress levels were rather low during the time of assessment or that the sAA reaction is modulated by a system other than the HPA axis. It could be that sAA is modulated by stressors that are short and acute in nature. This type of stress will elicit an initial quick response characterized by the fight-or-flight action; but once the stressor is chronic in nature, such as the case in chronic tinnitus, sAA is no longer affected but rather exhibits a blunt response. Consequently, this will allow the HPA axis to take over and produce cortisol in response to chronic stress.

Both sAA and cortisol seem to adapt to prolonged stress. The difference between them is that sAA reacts faster and for a short term while cortisol reacts slower and is longer in term. Future studies should further investigate the underlying mechanisms of sAA activity as a result of chronic pathologies, such as chronic tinnitus, and the relationship between cortisol and sAA as stress markers.

Melatonin

Melatonin is a biomarker produced by the pineal gland in darkness, and it has been found to be involved in the regulation of different functions including the circadian rhythm or internal time, modulation of blood pressure, and antioxidant activity (Prioda et

al., 2010; Paulis & Simk, 2007). The circadian rhythm is responsible for the regulation of sleep. As noted earlier, sleep disturbance is a frequent complaint of individuals with tinnitus (Alster et al., 1993).

Melatonin has also been found to have a favorable role in improving sleep in patients with tinnitus (Megwalu et al., 2006; Piccirillo, 2007). In addition to improving sleep, melatonin plays a role in microcirculation involving blood pressure (Neri et al., 2009) and functions as an antioxidant (Kim et al., 2009). Antioxidants in general, such as melatonin, play a role in protecting inner ear structures (Bas et al., 2009). Unfortunately, the underlying mechanisms of how melatonin functions in the inner ear, particularly in relation to tinnitus, is not well identified or investigated.

This study hypothesized that tinnitus subjects will experience a higher melatonin response and slower recovery time to a stressor than the controls. On the contrary, the findings of this study partially supported our hypothesis. There was a slight increase in melatonin levels in the tinnitus subjects from baseline to 5 minutes posttest; however, a more significant slower increase was observed post 30 minutes. This suggests that although melatonin did not exhibit a higher increase posttest, melatonin recovery time to baseline in the subjects with tinnitus was slower.

Overall, a rise in hormone levels at 5 minutes post stress was not observed in the subjects with tinnitus. These findings suggest evidence of a potential difference in the melatonin 5 min posttest values between the two groups. Studies have shown that sleep quality is significantly influenced by melatonin (Rosenberg et al., 1998). It is possible

that the observed lower levels of melatonin in this study's tinnitus group contributed to their overall poor sleep quality.

This data suggests that the normal reaction patterns for melatonin and cortisol for the control subjects were similar while the reaction patterns observed in the subjects with tinnitus differed. This could lead us to hypothesize that melatonin might exhibit the same pattern of function and/or activate the same networks as cortisol in the control group in this study but not in the tinnitus group. It is well documented that poor sleep and daytime sleepiness are common problems among subjects with tinnitus (Asplund, 2003). A possible explanation could be that since the tinnitus group was more sensitive to changes in their sleep pattern, their melatonin levels were also affected.

Most studies examine changes in melatonin intake over a longer period of time such as days or weeks. For example, the Rosenberg et al. (1998) study reported an improvement in tinnitus intensity with melatonin; however, they also found an improvement with a placebo. Furthermore, Rosenberg et al. (1998) found no statistical significance between the tinnitus handicap index (THI) and improvement in melatonin between the tinnitus and the placebo group. Similarly, this study found no statistical significance between melatonin levels and tinnitus severity index or the Pittsburgh sleep quality index, which was consistent with the findings of Uchechukwu et al. (2006). It is important to note that the current study used healthy controls for comparisons rather than a placebo group.

To date, few studies have investigated the effect and the underlying characteristics of melatonin on tinnitus. Future research should shed a light on the impact of melatonin

on sleep in subjects with tinnitus. This could possibly lead to safe treatment options for patients with tinnitus and sleep disorders.

Neopterin

Stress triggers the nervous system's active biomolecules such as cortisol and salivary alpha-amylase. These biomarkers have been identified to be involved in the modulation of stress as well as immune system reactions (Moynihan, 2003). Similar to the nervous system, the immune system also responds to chronic stress. A marker of the immune system reactivity to stress is neopterin. Neopterin occurs naturally in the body in oxidized form (Danova, 1998) and reflects stages of immune system activation and oxidative stress (Fuchs et al., 1988). Studies have shown that oxidative prolonged stress activates inflammatory reactions in the immune system and impacts different health conditions (Fuchs et al., 1988; Reibnegger et al., 1988; Flatow, Buckley, & Miller, 2013). Furthermore, the impact of oxidative stress has been indicated in noise-induced hearing loss (Henderson et al., 2006).

Taking into consideration the intensive nature of stress caused by chronic tinnitus, this study assumed that it would be of great benefit to examine the possibility of the role of the immune system in stimulating and modulating reactive inflammatory biomarkers in subjects with chronic tinnitus. We hypothesized that subjects with tinnitus will experience higher neopterin responses and slower recovery time to a stressor than controls.

The findings of this study revealed that levels of neopterin displayed a pattern different from the other three biomarkers. In the normal control group, neopterin levels

remained slightly unchanged from baseline through post-stress measurement times. Conversely, the neopterin levels were higher in the tinnitus group and essentially lowered rapidly but marginally after inducing the stressor. These findings could be used to indicate a potential difference at baseline between the two groups, control vs. tinnitus.

Higher levels of neopterin have been reported in other health conditions related to hearing such as noise- induced hearing loss (Henderson et al., 2006). Nevertheless, noise-induced hearing loss is among the most common known causes of tinnitus (Hiller & Goebel, 2006; Stouffer & Tyler, 1990; Martines et al., 2010). Similarly, 50% of this study's subjects with tinnitus regarded exposure to noise as the main cause of their tinnitus, while 50% reported stress to be the main cause of their tinnitus. We can make the assumption that in a population of subjects with tinnitus, where two of the primary tinnitus causes reported are noise exposure and stress, inflammatory reactions will be observed in reaction to the immune system stress-related biomarker, neopterin.

Studies have shown that higher levels of neopterin usually indicate a pro-inflammatory immune system (Chipitroil, 2010). One study by Marina et al. (2007) found that antioxidant therapy in patients with idiopathic tinnitus reduced oxidative stress and could probably reduce damage to the inner ear tissues. Perhaps one of the most significant findings of the Marina et al. study is that antioxidant therapy also reduces the subjective discomfort and intensity of tinnitus.

The findings of the current study suggest a potential involvement of the immune system in adapting to stress in subjects with chronic tinnitus. This study suggests that stress from chronic tinnitus may produce lower cortisol levels that reduce suppression of

the immune system and trigger inflammatory reactions exhibited in the higher neopterin values. Future research should further consider the role and underlying mechanisms of anti-inflammatory reactions of stress on highly stressed subjects with tinnitus.

Conclusion

This research study investigated the feasibility of a neural endocrinal immune model of investigating tinnitus in adult males with and without chronic tinnitus. Tinnitus is a complex disorder to investigate. Like other researchers, we who are concerned about the study of tinnitus are aware of the challenges that come with the study of such an intricate disorder. Tinnitus is multifaceted, lacking a clear identification of origin and mechanisms. Although we were not able to make any definitive statistical conclusions due to the small sample size of the current study, our findings support the feasibility of using a psychological immune endocrinal (T-NPIE) model of tinnitus. The higher neopterin values, for example, suggest that stress from chronic tinnitus produces lower cortisol, melatonin, and salivary alpha-amylase levels that suppress the immune system cells and trigger inflammatory reactions.

Limitations and Future Direction

Some of the weakness of this present study is the small sample size. In addition, considerations of each biomarker half time should have been considered, probably though the expansion of samples collection period. Future studies with larger sample sizes will provide data with more conclusive statistical analyses for each of the four stress hormones examined in this study. Once the role of these four stress-related hormones in the modulation of chronic tinnitus is known, additional studies of the effect of other

hormones and the hypothalamic-pituitary-adrenal (HPA) axis on tinnitus perception and modulation can be further considered. Additionally, tinnitus could be viewed as an inflammatory reaction. Future studies can also examine the role of other human hormones in chronic tinnitus. Additional physiologic methods and approaches to examine the T-PIE model would be to collect EEG data. FMRI and PET scan data could also examine predictions from the model. Future studies also need to examine the role of race in the perception of chronic tinnitus.

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APPENDIX A

MEDICAL HISTORY

Name: _____ Birthdate: _____
Last First Initial Month/ Day/ Year

1. Have you had any of the medical problems listed below? If you have not had the problem, CIRCLE (No). If you have had the problem CIRCLE (Yes). Please fill in the blank showing how old you were when the problem began. Also, indicate whether you have the problem now.

Have you had...			**About how old were you when problem began?**	**Do you have this problem now?**	
Heart disease	No	Yes	_____years old	No	Yes
High blood pressure	No	Yes	_____years old	No	Yes
Hardening of arteries (arteriosclerosis)	No	Yes	_____years old	No	Yes
Varicose veins, phlebitis	No	Yes	_____years old	No	Yes
Stroke	No	Yes	_____years old	No	Yes
Emphysema, asthma	No	Yes	_____years old	No	Yes
Arthritis or rheumatism	No	Yes	_____years old	No	Yes
Diabetes	No	Yes	_____years old	No	Yes
Thyroid problem	No	Yes	_____years old	No	Yes
Kidney disease	No	Yes	_____years old	No	Yes
Cancer	No	Yes	_____years old	No	Yes
Depression	No	Yes	_____years old	No	Yes

Do you have **other** significant health problems? No ..... 1 Yes 2
IF **YES**: Please list problems below:

_____	_____years old	No	Yes
_____	_____years old	No	Yes
_____	_____years old	No	Yes
_____	_____years old	No	Yes

(Please use an extra sheet of paper if you need more space for any questions on this page.)

2. What medications are you taking **currently?**

Medication name:	**Taken for what condition:**	**Started about when:**
_____	_____	_____ years old
_____	_____	_____ years old
_____	_____	_____ years old
_____	_____	_____ years old

3. Have you had surgery for any reason? No.....1 Yes.....2

Surgery #1: _____ at about _____years old
Surgery #2: _____ at about _____years old
Surgery #3: _____ at about _____years old

4. Have you been hospitalized for severe burn, wound, or other serious medical problems? No.....1 Yes.....2

Problem #1: _____ at about _____years old
Problem #2: _____ at about _____years old

Adapted from Meikle, Griest & Press (1986) 11.10.94

5. How often do you take over-the-counter medications for pain, headache or arthritis?	Never or almost never	Once a week or less	Several days each week	Once a day	More than once each day
A. Aspirin (Anacin, Excedrin, Empirin, Ascriptin)	1	2	3	4	5
B. Ibuprofen (Advil, Nuprin, Motrin)	1	2	3	4	5
C. Acetaminophen (Tylenol, Datril)	1	2	3	4	5
D. Other pain medication: _____	1	2	3	4	5
E. Other pain medication: _____ *(Name of medication)*	1	2	3	4	5

6. If you have tinnitus, have any medications caused your tinnitus to change in any way?
   - No 1
   - Yes 2
   - Do not have tinnitus 3

   If **YES**, list medications that caused tinnitus changes and describe how tinnitus changed:
   _____
   _____
   _____

7. How often do you get headaches?
   - Rarely 1
   - Several per month 2
   - Several per week 3
   - Daily 4

8. Are headaches a significant problem for you?
   - Not a problem 1
   - Small problem 2
   - Moderate problem 3
   - Big problem 4
   - Very big problem 5

9. Have you had any problems with your teeth or jaw?
   - No ..... 1 *(Go on to Q.10)*
   - Yes 2

   9a. Pain or discomfort of jaw ..... No Yes
   9b. Incorrect bite or other misalignment ..... No Yes
   9c. Jaw injury, surgery, infection ..... No Yes
   9d. Clicking or other noise in jaw ..... No Yes

   9e. When did you **start** having problems with your teeth or jaw? _____ years old

   9f. Are you currently having problems with your teeth or jaw? ..... No Yes

10. If you have tinnitus, does it **change** when you move your jaw or clench your teeth?
    - No change 1
    - Tinnitus gets louder 2
    - Tinnitus gets softer 3
    - Tinnitus changes in other way(s) 4
    - Do not have tinnitus 5

11. How often have you needed medical care during the past 6 months? For each question, please CIRCLE the answer that best describes you:	Never	Once	Two-three times	More than three times
During the past 6 months...				
A. How many times were **you** admitted as a patient in a **hospital**?	1	2	3	4
B. How many times did **you** receive treatment at an **emergency room or urgent care center**?	1	2	3	4
C. How many separate times did **you** need to visit a **medical doctor or clinic** (not counting hospital, emergency room or urgent care)?	1	2	3	4

5-10: Adapted from Meikle, Griest & Press (1986); 11: Adapted from Kaiser Permanente, NW Region (undated)

11.10.94

HEALTH AND DAILY LIFE

Because of a physical or health problem, do you have any difficulty when you do the following activities by yourself and without using special equipment?	HOW MUCH DIFFICULTY? NO DIFFICULTY	SOME	A LOT	UNABLE TO DO IT
1. Preparing your own meals	1	2	3	4
2. Shopping for personal items (such as toilet items or medicines)	1	2	3	4
3. Managing your money (such as keeping track of expenses, or paying bills)	1	2	3	4
4. Using the telephone	1	2	3	4
5. Doing heavy housework (like scrubbing floors, or washing windows)	1	2	3	4
6. Doing light housework (like dishes, straightening up, or light cleaning)	1	2	3	4

People sometimes have problems doing their normal daily activities if they have chronic, long lasting health problems of any type, including hearing problems or tinnitus.

When you think back over the past 6 months, have you had any trouble doing your work or other regular daily activities, as a result of problems with your hearing, or tinnitus, or your overall health?

In the past 6 months, have you found that you...

7. Had to take **frequent rests** when doing work or other activities	No	Yes
8. Cut down the **amount of time** you spent on work or other activities	No	Yes
9. **Accomplished less** than you would like	No	Yes
10. Did not do work or other activities as **carefully** as usual	No	Yes
11. Were limited in the **kind** of work or other activities	No	Yes
12. Had **difficulty** performing work or other activities (for example, it took extra effort)	No	Yes
13. Required **special assistance** (the assistance of others, or special devices)	No	Yes

14. Are you currently employed?
    YES,
    - Employed full-time 1
    - Employed part-time or on-call 2

    NO,
    - Retired 3
    - Looking for employment 4
    - Unemployed because of health 5
    - Other reason: 6

    _____
    (Please describe)

15. What kind of work have you done most of your working life?_____
    _____

16. What is your current marital status?
    - Married, living with spouse 1
    - Married, separated 2
    - Widowed 3
    - Divorced 4
    - Never married 5

17. Compared to other persons your age, would you say that your health is:
    - Excellent 1
    - Very good 2
    - Good 3
    - Fair 4
    - Poor 5

1-6: Adapted from 1984 National Health Interview Survey, Supplement on Aging; 7-13,17:Adapted from RAND (1986);14:Adapted from Neal (1990);15&16:Stewart & Archibold (1994) 11.10.94

The following questionnaire lists some problems people sometimes have, particularly if they have any type of chronic health condition. Even if you do not have any chronic health conditions, it would help us to know which of these problems might apply to you.

When trying to decide whether a statement applies to you, THINK BACK OVER THE PAST 6 MONTHS. If the statement is true for you, CIRCLE one of the numbers on that same line, to indicate the correct description. If it does not apply to you over the past 6 months, select "1" (to indicate "not at all").

HOW MUCH DOES THE STATEMENT APPLY TO YOU?	NOT AT ALL	A LITTLE	A MODERATE AMOUNT	QUITE A BIT	VERY MUCH
1. I have difficulty falling asleep	1	2	3	4	5
2. I have difficulty staying asleep	1	2	3	4	5
3. My appetitite is poor	1	2	3	4	5
4. I am not able to work	1	2	3	4	5
5. I lose too much work time because of health problems	1	2	3	4	5
6. I am not able to perform all of my duties at home or at work because of health problems	1	2	3	4	5
7. I have difficulty concentrating	1	2	3	4	5
8. I have difficulty remembering	1	2	3	4	5
9. I have difficulty thinking clearly	1	2	3	4	5
10. I have difficulty doing household chores	1	2	3	4	5
11. I have difficulty with transportation	1	2	3	4	5
12. It is hard for me to get out of the house very much	1	2	3	4	5
13. I am sitting or lying down most of the day	1	2	3	4	5
14. I have difficulty enjoying time with relatives and/or friends	1	2	3	4	5
15. I have problems in planning social activities because I do not know how I will feel	1	2	3	4	5
16. I have difficulty going out to dinner, movies and other activities	1	2	3	4	5
17. Family or friends do not come over to visit often	1	2	3	4	5
18. I do not get along well with my family	1	2	3	4	5
19. It has been difficult to maintain old friendships	1	2	3	4	5
20. I find it difficult to meet new friends	1	2	3	4	5
21. My family expects me to do more than I am capable of doing	1	2	3	4	5
22. I have difficulty relaxing	1	2	3	4	5
23. I feel irritated or nervous quite often	1	2	3	4	5

Adapted from Kames, Naliboff, Heinrich & Schag (1984)

11.10.94

APPENDIX B

TINNITUS HISTORY

Appointment

Name_____ Age____ Date:_____ Time_____

Address_____

Birthdate _____ Phone (____)_____-_____ (____)_____-_____
Month/ Day/ Year *Home* *Work*

Male ❑ Female ❑ Eye Color:_____ Your Preferred Hand: Right ❑ Left ❑ Unsure ❑

Referred to Tinnitus Clinic by:_____

2. About how long have you been aware of having tinnitus?
   1 .....Less than 1 yr 4 .....6 - 10 years
   2 .....1 - 2 years 5 .....11 - 20 years
   3 .....3 - 5 years 6 .....20+ years

3. Some people know the date when their tinnitus started. *YY/MM/DD if known*:_____

4. Did you become aware of your tinnitus suddenly or more gradually?
   Suddenly (1 week or less) 1
   More gradually 2
   Do not know 3

5. Were illness, accident or other special circumstances associated with the onset of your present tinnitus? *(Please describe briefly)*
   _____
   _____

5a. Before that did you experience any episodes of temporary or milder tinnitus?
   No 1
   Yes 2

5b. If **YES**, circle all that apply

   After exposure to loud sound 1
   Associated with colds, flu, or allergies 2
   Any other time(s): _____
   _____

6. Since it started, has there been any change in the **amount of time** you are aware of hearing tinnitus?
   No, there has been no change 1
   Yes, I now hear tinnitus **more** of the time 2
   Yes, I now hear tinnitus **less** of the time 3
   I am not sure if the amount of time I hear it has changed 4

7. Which **one** of the statements below best describes your current tinnitus?

   Tinnitus usually lasts a few minutes at most 1
   Tinnitus usually lasts up to several hours 2
   Tinnitus usually lasts up to several days 3
   Tinnitus is always there 4

8. If your tinnitus is **not** present all the time, about how much of the time does it seem to be present?

   Less than half the time 1
   Half the time or more 2

9. How **much** of a **problem** is your tinnitus?
   Not a problem 1
   A small problem 2
   A moderate problem 3
   A big problem 4
   A very big problem 5

9a. If tinnitus **is** a problem, about **how long** has your tinnitus been a problem?
   1 year or less ..... 1 *(Go on to Q.10)*
   More than 1 year 2

9b. If more than 1 year, about **how many** years?
   _____years

10. Which is **more** of a problem for you, **hearing difficulty**...or...**tinnitus**?

   Hearing difficulty is worse problem ... 1
   Tinnitus is worse problem 2
   They are equally bothersome 3
   Not sure 4
   Neither one is a problem 5

Adapted from Meikle, Griest & Press (1986) 11.10.94

11. Where does your tinnitus appear to be located?

A. **Left** ear	No	Yes
B. **Right** ear	No	Yes
C. **Both** ears	No	Yes
D. **In head**, on **left** side	No	Yes
E. **In head**, on **right** side	No	Yes
F. **Fills head**	No	Yes
G. Other location	No	Yes

_____
(Please describe)

12. If your tinnitus is in more than one location, where is it **worst**?
(**CIRCLE** only **One** answer below)

Left ear worst 1
Right ear worst 2
Both ears **equal** 3
In head, left side worst 4
In head, right side worst 5
Fills head 6
Other location 7

_____
(Please describe)

13. Since it started, has the **location** of your tinnitus changed?

No change 1
Started in 1 ear, now in both 2
Started in both ears, now in 1 ear 3
Other 4

_____
(Please describe changes)

14. Do you feel that tinnitus makes it more difficult to hear clearly?

No ..... 1 *(Go on to Q.15)*
Sometimes 2
Often 3
Unsure 4

14a. If tinnitus does make it more difficult to hear, in what situations does tinnitus interfere with your hearing?

_____
_____
_____
(Please describe)

15. Does your tinnitus seem to be one sound or more than one sound?

1 sound 1
2 sounds 2
3 or more sounds 3
Unsure 4

16. In the list below, please choose the sound or sounds that most closely resemble your tinnitus.

A. Ringing	No	Yes
B. Clear tone	No	Yes
C. More than one tone	No	Yes
D. Whistle	No	Yes
E. Hissing	No	Yes
F. Buzzing	No	Yes
G. Hum	No	Yes
H. Music	No	Yes
I. Sizzling	No	Yes
J. Transformer noise	No	Yes
K. High tension wire	No	Yes
L. Crickets, insects	No	Yes
M. Pulsating	No	Yes
N. Pounding	No	Yes
O. Ocean roar	No	Yes
P. Clicking	No	Yes

Q. Other: _____
_____

17. From the list above, or in your own words, please LIST your tinnitus sound(s) starting with the sound that bothers you the most:

	Sounds like:		Location:
1.	_____	is in:	_____
2.	_____	is in:	_____
3.	_____	is in:	_____
4.	_____	is in:	_____

18. Besides the sounds that you listed above, do you hear any additional tinnitus sounds?

No, I don't ever hear any other tinnitus sounds 1
Yes, I sometimes hear other tinnitus sounds 2
Yes, I hear additional sounds most or all of the time 3

Adapted from Meikle, Griest & Press (1986)

11.10.94

19. On the scale below please (CIRCLE) the number that best describes the loudness of your **usual** tinnitus:

1	2	3	4	5	6	7	8	9	10
Very quiet				Intermediate					Very loud

20. Since it started has your tinnitus grown **louder** than when you first noticed it?

- No change in loudness 1
- Tinnitus has grown louder 2
- Tinnitus has grown softer 3
- Both types of change have occurred 4
- Not sure if loudness of tinnitus has changed 5

21. Does the **loudness** of your tinnitus tend to fluctuate up and down?

- Loudness rarely or never fluctuates 1
- Loudness fluctuates several times per month 2
- Loudness fluctuates several times per week 3
- Loudness fluctuates daily 4

21a. If your tinnitus shows **loudness fluctuations**, how large are the changes usually?

- Barely noticeable 1
- Moderate 2
- Very marked 3
- Variable in size 4

22. Have you noticed an increase in tinnitus **loudness** caused by any of the following?

A. Noise exposure	No	Yes
B. Stress or fatigue	No	Yes
C. Colds, sinus, hayfever	No	Yes
D. Tobacco use	No	Yes
E. Alcohol use	No	Yes
F. Aspirin or other pain medication	No	Yes
G. Head or neck injury	No	Yes

22a. Anything else that has caused changes in your tinnitus? (Please indicate whether tinnitus became louder or softer)

_____

_____

23. Does your tinnitus interfere with sleep?

- No ..... 1 *(Go on to Q.24)*
- Yes, sometimes 2
- Yes, often 3

23a. If tinnitus does cause you sleep problems, how severe is the problem?

- Mild problem 1
- Moderate problem 2
- Severe problem 3

23b. Have you found anything that helps you sleep?

_____

_____

(Please describe)

24. How much of an effort is it for you to **ignore** tinnitus when it is present?

- Can easily ignore it 1
- Can ignore it with some effort 2
- It takes considerable effort 3
- Can never ignore it 4

25. How much **discomfort** do you usually experience when your tinnitus is present?

- No discomfort 1
- Mild discomfort 2
- Moderate discomfort 3
- A great deal of discomfort 4

26. Have you changed jobs because of tinnitus?

- No, tinnitus has not caused job change(s) 1
- Yes 2

_____

_____

(Please describe job changes)

27. Have you made other changes in your lifestyle because of tinnitus?

- No, tinnitus has not caused lifestyle change(s) 1
- Yes 2

_____

_____

(Please describe lifestyle changes)

Adapted from Meikle, Griest & Press (1986)

11.10.94

APPENDIX C

UNCG TINNITUS CLINIC TINNITUS SEVERITY INDEX

The problems listed below are sometimes reported by people with tinnitus. How often has tinnitus caused you to have the problems listed below?

	Never	Rarely	Sometimes	Usually	Always
Has tinnitus....					
1. Made it uncomfortable to be in a quiet room?.....	1	2	3	4	5
2. Made you feel irritable or nervous?.....	1	2	3	4	5
3. Made you feel tired or stressed?.....	1	2	3	4	5
4. Made it difficult for you to relax?.....	1	2	3	4	5
5. Made it difficult to concentrate?.....	1	2	3	4	5
6. Made it harder to interact pleasantly with others? ...	1	2	3	4	5
Does tinnitus....					
7. Interfere with your social activities or other things you do in your leisure time?.....	1	2	3	4	5
8. Interfere with your required activities (work, home care, other types of responsibilities)?.....	1	2	3	4	5
9. Interfere with your overall enjoyment of life?.....	1	2	3	4	5
10. Interfere with your ability to sleep?.....	1	2	3	4	5
11. Is it difficult for you to ignore your tinnitus?.....	1	2	3	4	5
12. How often do you experience discomfort from tinnitus?.....	1	2	3	4	5

Adapted from Meikle, Griest & Press (1986) 12/11/01

APPENDIX D

PERCEIVED STRESS SCALE

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Name _____ Date _____

Age _____ Gender (*Circle*): **M F** Other _____

	0 = Never	1 = Almost Never	2 = Sometimes	3 = Fairly Often	4 = Very Often
1. In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5. In the last month, how often have you felt that things were going your way?	0	1	2	3	4
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
7. In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
8. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9. In the last month, how often have you been angered because of things that were outside of your control?	0	1	2	3	4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Please feel free to use the *Perceived Stress Scale* for your research.

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References

The PSS Scale is reprinted with permission of the American Sociological Association, from Cohen, S., Kamarck, T., and Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24,* 386-396.

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APPENDIX E

PITTSBURGH SLEEP QUALITY INDEX

Subject's Initials_____ID#_____Date_____Time_____AM PM

PITTSBURGH SLEEP QUALITY INDEX

INSTRUCTIONS:
The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, what time have you usually gone to bed at night?

   BED TIME _____

2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

   NUMBER OF MINUTES _____

3. During the past month, what time have you usually gotten up in the morning?

   GETTING UP TIME _____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)

   HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you . . .

a) Cannot get to sleep within 30 minutes

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

b) Wake up in the middle of the night or early morning

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

c) Have to get up to use the bathroom

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

d) Cannot breathe comfortably

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

e) Cough or snore loudly

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

f) Feel too cold

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

g) Feel too hot

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

h) Had bad dreams

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

i) Have pain

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

j) Other reason(s), please describe_____

_____

How often during the past month have you had trouble sleeping because of this?

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

6. During the past month, how would you rate your sleep quality overall?

Very good _____

Fairly good _____

Fairly bad _____

Very bad _____

APPENDIX F

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

APPENDIX G

SUPPLEMENTARY METHODS

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APPENDIX G

SUPPLEMANTRY METHODS

Salivary Cortisol Determination

Saliva samples will be assayed in duplicate to determine cortisol levels using a highly sensitive enzyme immunoassay (Salimetrics, State College, PA). The test uses 25 µL of saliva per determination, has a lower limit of sensitivity of 0.007 µg/dL, standard curve range from 0.012 µg/dL to 3.0 µg/dL, an average intra-assay coefficient of variation of 4.6% and an average inter- assay coefficient of variation of 5.9%. Method accuracy determined by spike and recovery averaged 105.3% and linearity determined by serial dilution averaged 105.3%. Values from matched serum and saliva samples show the expected strong linear relationship, $r(47) = 0.91$, $p < 0.0001$.

Salivary Melatonin Determination

The melatonin assays were performed as outlined in the manufacturers' protocol using a highly sensitive enzyme immunoassay (BÜHLMANN Direct Saliva Melatonin ELISA; Cat# EK-DSM) at Salimetrics, State College, PA. The test uses 200 µl of saliva per determination, has a lower limit of sensitivity (analytical sensitivity) of 0.50 pg/mL, functional sensitivity of 1.6 pg/mL, standard curve range from 0.6 pg/mL to 25 pg/mL, an average intra-assay coefficient of variation of 12.6% and an inter-assay coefficient of variation of 22.9%. Method accuracy determined by spike recovery averaged 97.9% and linearity determined by serial dilution averaged 92.2%.

Salivary Alpha-amylase Determination

All samples will be assayed for sAA using a commercially available kinetic reaction assay (Salimetrics, State College, PA). The amount of sAA activity present in the sample is directly proportional to the increase (over a 2 min period) in absorbance at 405 nm.Saliva samples (10 µL) are diluted 1:200 in assay diluent and well mixed. 8 µL of diluted sample or control are then pipetted into individual wells of a 96-well microtiter plate. 320 µL of preheated (37°C) chromagenic substrate solution (2-chloro-p-nitrophenol, linked to maltotriose) is added to each well and the plate is rotated at 500-600 RPM at 37 °C for 3 minutes. Optical density (read at 405 nm) is determined exactly at the one-minute mark and again at the 3-minute mark

Salivary Neopterin Determination

All samples were tested for salivary neopterin in duplicate using a highly-sensitive enzyme immunoassay (performed at Salimetrics LLC, State College PA). The test used 50µl of saliva per determination. The assay has a lower limit of sensitivity of 0.51 ng/mL, range of standard curve; 0.51 to 25.3 ng/mL. The average intra-and inter-assay coefficients of variation are 3.5 % and 5.4 % respectively. Assay Characteristics: method accuracy determined by spike recovery was found in an acceptable range (99-110%) and linearity determined by serial dilution averaged 105.1 %.